Sol Gel Method Performed for Biomedical Products Implementation

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Abstract: Sol–gel technology is an impressive and instructive innovation in science that necessitates a multidisciplinary approach for its important applications in the practice. An important peculiarity of the sol gel technology is the possibility to control the mechanism and kinetics of the chemical reactions, thus monitoring the final structure (particle size, porosity, thin layer thickness) of the materials. The low processing temperature combined with the intrinsic bio-compatibility and environmental friendliness of the implied components makes it an ideal method applied in different biomedical purposes: the synthesis of porous matrices for entrapping of organic and inorganic compounds, sensor molecules, enzymes and many other biological molecules, selective coatings for optical and electrochemical biosensors, stationary phases for chromatography, immunoadsorbent and solid-phase extraction materials, controlled release agents, solid-phase biosynthesis, and unique matrices for biophysical studies. It is therefore the scope of this review to provide a few insights of the recent progresses made in sol-gel-based materials for biomedical applications.

Keywords: Bioencapsulation, biosensor, drug delivery, hybrid composite, medical devices, sol-gel technology.

1. INTRODUCTION

Advanced preparation methods for materials with new features are feasible on the basis of colloid-chemical processes and nanochemistry. In this respect, the sol-to-gel transformation at molecular level and low temperature, followed by solidification and chemical modification is of great interest to attain new materials with non-traditional physical, chemical and functional properties of special interest.

The typical sol - gel method as a wet-chemical technique for the obtainment of materials starts with a chemical solution ("sol" short for solution) or colloidal particles ("sol" for nanoscale particle) which acts as the precursor, consisting of metal compounds as source of oxides, water and hydrolysis agent, alcohol as solvent and acid or base catalyst. Metal compounds undergo hydrolysis and polycondensation at near room temperature, giving rise to sol, in which polymers or fine particles are dispersed. Further reaction connects the particles, solidifying the sol into an integrated network or a wet gel, which contains water and solvents.

The method allows the formation of materials with different configurations (monoliths, thin films, fibers, powders). The great diversity of materials makes the process very attractive in many domains of applications: optical, electrical and electronic, biomaterials, sensors, separation (chromatographic).

The distinction between the sol-gel process and the traditional methods of materials' forming is:

- High chemical homogeneity and purity component, containing in the final material on a molecular scale;
- Flexibility and controllability of the process;
- Forming of silicate and ceramic matrices at lower temperatures.

The sol-gel technology is versatile, simply, cheaply and ecologically. There is believed as energy and resource saving process. One more advantage is the simplicity of the necessary equipment, too [1].

Gupta [2, 3] has also underlined, the sol-gel technology constitutes an innovative way in science that requires a multidisciplinary approach for its various applications. The reaction is easy to perform, does not require special conditions (can be done on the bench top in a beaker), and offers the possibility of various forming processes. At the same time, there are recognized the improvements in the ability to control/tailor the whole process, the reproducibility and the control of porosity, as well as the greater automation, the ability to control phase development in films.

Another unique feature of the process is the monitoring of pore–solid architecture. There is extraordinary control not only of the size (mesopores of 2–50 nm) but also the arrangement of pores within the inorganic (or organic/inorganic) framework. The design of materials with specific architectures is enabling researchers to obtain unique properties in such diverse areas as drug delivery and electrochemistry.

By applying the sol-gel technique, a significant impact in modification of the materials properties can be achieved (Fig. **1**): mechanical (modulus, strength, toughness, impact, fracture, fatigue, abrasion, scratch), thermal (degradation, glass transition Tg), chemical (degradation, solvent resistance), permselectivity, optical, electrical.

Potential applications of sol–gel technology in the areas of defense, nanotechnology, environmental monitoring and biomedical devices are now continuously emerging [4-7].

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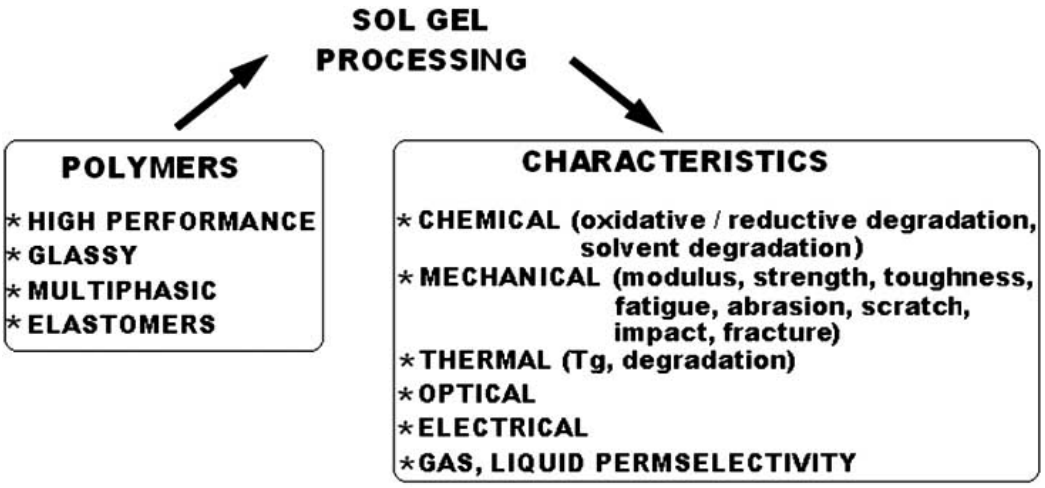


Fig. (1). Sol-gel process applications in the material modification.

2. BASIC CONCEPTS IN SOL – GEL SYNTHESIS

The synthesis of SiO_2 from liquid silicon metal–organic precursors is probably the oldest and most investigated sol–gel process. Typically, it involves hydrolysis and condensation of a metal–organic precursor, such as tetraethoxysilane, in an appropriate solvent, such as ethanol, with or without the use of a catalyst. As these reactions proceed and the viscosity of the solution increases, a gel is formed. It is typically made of Si–O–Si bonds, forming a network within which reaction products are trapped. A nanoporous structure results when such products are removed *via* evaporation. Pore size, distribution, and interconnectivity are affected by processing parameters, such as the type and amount of solvent and/or catalyst, temperature, and in some cases, the presence and arrangement of templating molecules. By themselves, these porous gels constitute a very interesting class of nanomaterials. The nanoporous architecture may be tailored for connectivity, orientation, arrangement, or size and exploited for various applications, such a chromatography columns, sensors, catalyst supports, low dielectric constant materials, or controlled release of reactants.

Over the past several decades the sol-gel method has a remarkable growth. Also, a number of reviews on sol–gel technology have appeared with specific applied areas [8 – 14].

The method schematically represented in Fig. (**2**) enables the formation of gels with different configurations, as a function of the used drying:

- drying in mild conditions: the gel hardens and becomes compacted: xerogel (glasses and dense ceramics);
- solvent evaporation in supercritical conditions: the formation of a less compact gel named aerogel;
- spreading the sol on a surface to obtain thin films of xerogel (by different coating techniques).

Initially, the sol gel technologies were developed during many years as an alternative for the preparation of glasses and ceramics at considerably lower temperatures. Sol-gel processing is sufficiently flexible, that it is particularly appealing to material scientists in the production of a wide range of highly porous inorganic oxide and carbon based networks. The initial system represents a solution where different polymerization and polycondensation processes lead to the gradual formation of the solid phase network.

Based on the scientific investigations, many routes of synthesis have been investigated. The schemes of synthesis are dependent of the initial precursors: aqueous solutions of metal salts; metal alkoxide solutions; mixed organic and inorganic precursors. The process may be realized by alkoxide route and by colloidal route.

The strategy to construct hybrid materials consists of making intentionally strong bonds (covalent or iono-covalent) between the organic and inorganic components. The inorganic polymerization proceeds by hydrolysis of the alkoxide precursors to introduce a reactive hydroxyl group on the metal. This step is followed by the formation of metal oxo-bridges or metal hydroxo-bridges by condensation or addition reaction, respectively. The formation of hydroxo-bridges occurs when the coordination number of the metal is higher than the valence state. Alkoxides are not miscible with water; a common solvent usually an alcohol is used. The oxo metallic network progressively grows from the solution, leading to the formation of oligomers, oxopolymers, colloids (sols or gels) and a solid phase.

Sol-gel reactions promote the growth of colloidal particles (sol) and their subsequent network formation (gel) through the hydrolysis and condensation reactions of inorganic alkoxide monomers [15]. The precursors for synthesizing these colloids consist of a metal or metalloid element surrounded by various reactive ligands. Metal alkoxides are most popular because they react readily with water. The most widely used metal alkoxides are the alkoxysilanes, such as tetramethoxysilane (TMOS) and tetraethoxysilane (TEOS). However, other alkoxides such as aluminates, titanates, zirconates, and borates are also commonly used in the sol-gel process, either alone or in combination with other alkoxides, such as TEOS.

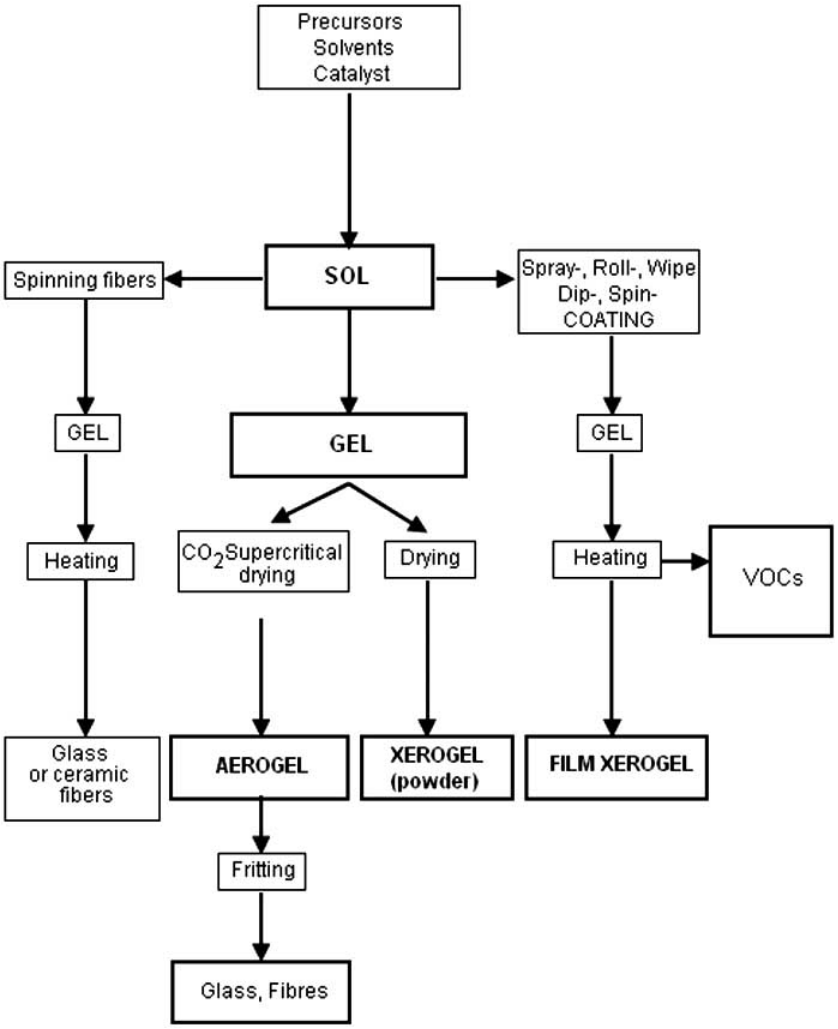


Fig. (2). Schematic diagram of the sol-gel technique in the material processing.

A kind of sol-gel reaction based on hydrolysis and condensation of an alkoxysilane, is presented in Fig. (**3**) [15]. Hydrolysis is initiated by the addition of water to the TEOS solution under acidic, neutral, or basic conditions.

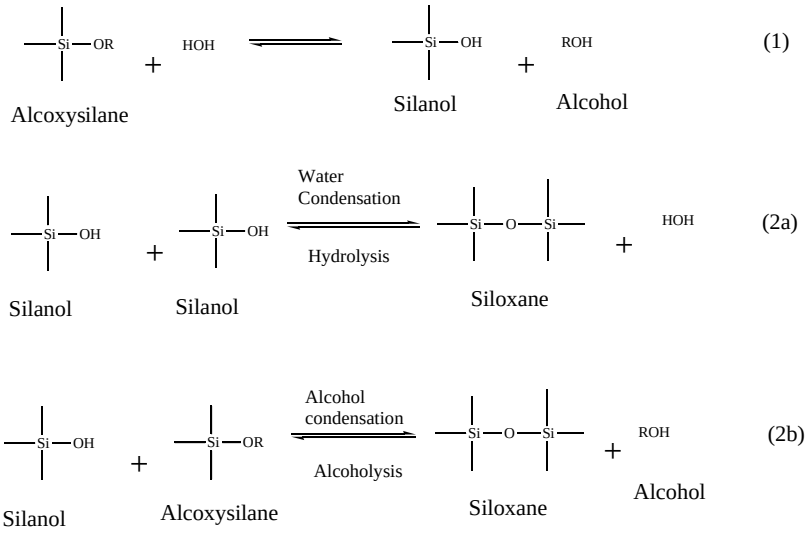


Fig. (3). Sol – gel process: the general reaction scheme [15].

The reactions of alkoxysilanes can be summarized in terms of three steps: hydrolysis of the alkoxide, silanol-silanol condensation, and silanol-ester condensation. The hydrolysis occurs by the nucleophilic attack of oxygen contained in water in the silicon atom. Subsequent condensation reactions take place producing siloxane bonds. The polymerization stages may be described as, (1) polymerization of monomers to polymers, (2) condensation of polymers to primary crystals, (3) growth or agglomeration of primary crystals to particles and (4) linking of particles into chains and to three dimensional network [1].

Networks of the chains extend throughout the liquid medium, thickening the network into a gel. In the last stage water and alcohol are evacuated from the network structure causing gradual shrinkage and even cracking of the monolithic gel.

Like it is already underlined, in the most sol-gel conditions the Si-C bond remains stable towards hydrolysis and the R' group introduces focused new properties to the inorganic network (flexibility, hydrophobicity, refractive index modification, optical response, etc).

Organic groups R' can be introduced into an inorganic network in two different ways: as network modifiers or network formers. Both functions have been achieved in the so-called ORMOCER®s - organically modified ceramics - (registered trademark of Fraunhöfer-Gesellschaft zur Förderung der angewandten Forschung e.V. in Germany). Since the eighties, these products have been extensively studied and developed by the Fraunhöfer Institut für Silicatforschung, Würzburg [16-19]. The organic group R' can be any organofunctional group. If R' is a simple nonhydrolizable organic group (Si-CH_3, Si-phenyl, etc) it will have a modifying effect. Moreover if R' can react with itself (R' contains a vinyl, a methacrylic or an epoxy group) or additional polymerizable monomers, it acts as a network former. Sanchez *et al.* [20] were gathered examples of network formers and network modifiers. Polymeric components can also be introduced in the hybrid nanocomposites by using functionalized macromonomers (R'') of general formula:

$$(OR)_{4-n}Si - R'' - Si(OR)_{4-n} \qquad \text{with} \quad n = 1, 2, 3$$

Whereas most of the investigations were carried out in organic solution due to the solubility of alkoxides in these solvents, aqueous route or *colloidal route* also was investigated, especially if one moves to the sol phase, because inorganic sols (stabilized colloids) were known since a long time, especially in the case of silica [1].

2.1. Factors Influencing the Sol-Gel Reaction

A number of conditions can influence the hydrolysis and condensation reactions. Of these, the most relevant include water-to-alkoxide ratio, type and amount of catalyst, type of network modifier, and solvent effects [3, 15, 21].

The effect of the water / alkoxide ratio for the sol-gel process is such, that as the ratio increases, so does the SiO_2 content of the gel [15]. Therefore, for complete hydrolysis, there must be at least one mole of water for every alkoxide group. However, in a recent article by McCormick *et al.* [22], whose experiments were conducted over a wide range of water / TEOS ratios, there was no correlation between the water / alkoxide ratio and the achievement of complete hydrolysis. The validity of these experiments on the effect of water / alkoxide ratio is correctly because water is *in situ* generated in the reaction and therefore the reaction, once catalyzed, self-propagates the hydrolysis.

One important subject for consideration when deciding the concentration of catalyst is whether a precise concentration is needed [15]. In the sol-gel process, water is *in situ* generated through condensation reactions. This makes difficult the addition of a precise amount of catalyst. In addition to water / TEOS ratio experiments, McCormick *et al.* [22] synthesized sol-gel films, adding a wide range of acid concentrations. Their results indicated no correlation between the acid concentration and acid initiation of the sol-gel reaction. The results show that a minimum catalytic amount of acid is necessary in all the experiments for the self propagation of the reaction. The kinetic of the reaction could be modified, but not the basic structure of the overall network.

Hydrolysis and condensation reactions of most inorganic alkoxides can be carried out without catalyst because of the extremely fast rates of reaction. However, alkoxysilanes hydrolyze more slowly, requiring the addition of either an acid or base catalyst [15] (Fig. **4**). Acid-catalyzed reactions, having a particle nucleation rate-determining process, tend to yield more linear-like networks due to the fast hydrolysis. Therefore, acid catalyzed systems have a less completely formed network of siloxane bonds with a higher concentration of unreacted silanols. Base-catalyzed reactions, on the other hand, yield highly dense materials due to the longer time that the sol particles have to aggregate and arrange themselves in the most thermodynamically stable arrangement.

In a presentation [15] - not to scale - in Fig. (**5**), there are some pH-dependent rate profiles for the hydrolysis and condensation reactions; thus, there is observed [3, 15, 16-19, 23] the reaction rates are in essence dependent on pH: at pH = 7, hydrolysis occurs at a slow rate, while condensation occurs at a fast rate. It is this inverse correlation between the rates of the hydrolysis and condensation reactions that controls the kinetics of the reactions and as a result, the final network structure.

Also, the presence of organic groups determines the coordination centers with functionality less than four and influences the reactivity of the alkoxy groups and therefore the connectivity of the sol-gel network in two ways [15]:

(1) Formation of siloxane bonds requires the diffusion of partially hydrolyzed molecules. The larger the alkyl group attached to the silicone, the slower the rate of diffusion and therefore the less interconnection within the network.

(2) It is also reported that larger alkyl groups produce polymers with higher surface area [3]. Larger surface area allows for higher unreacted oxide concentration in the gels. This produces a branching effect in the sol-gel network. At the same time, the presence of bulky and/or long alkyl substituents, will affect the

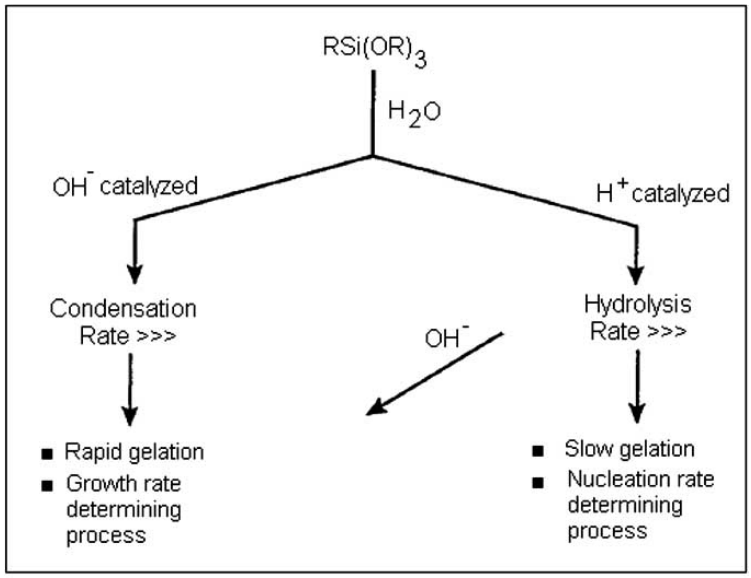


Fig. (4). Effect of catalyst on hydrolysis and condensation [15].

rate of the hydrolysis reaction by hindering the inversion of the S_N2 transition state [15, 24].

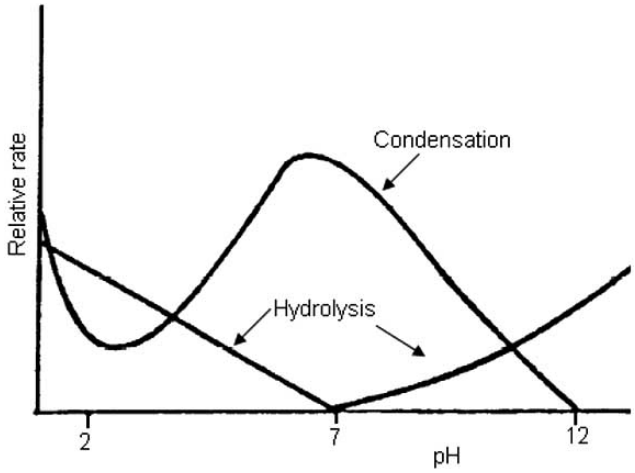


Fig. (5). Effect of pH on hydrolysis and condensation rates.

The effect of solvents on hydrolysis and condensation reactions is not usually discussed primarily because solvents other than water and simple alcohols, which are produced internally, are not often used. However, addition of external solvents can have an important effect on controlling hydrolysis and condensation rates [15].

3. BIOENCAPSULATION OF BIOMOLECULES BY SOL GEL TECHNIQUE

The encapsulation or generation of new surfaces that can fix biomolecules firmly without altering their original conformations and activities is still challenging. The sol–gel chemistry offers new and interesting possibilities for the promising encapsulation of heat-sensitive and fragile biomolecules (enzyme, protein, antibody and whole cells of plant, animal and microbes) because it is an inherent low temperature and biocompatible process [25]. In aqueous solutions, biomolecules such as enzymes, antibodies, proteins, lose their functionality. These problems can be minimized considerably by biomolecules immobilization. Taking into account these advantages, since the 1960s, an extensive variety of techniques have been developed to immobilize biomolecules, including adsorption, covalent attachment and entrapment in various polymers [25]. Generally, adsorption techniques are easy to perform, but the bonding of the biomolecules is often weakly and such biocatalysts lack the degree of stabilization and easy leakage from the matrix. The covalent linkage method increases the stability but often requires several chemical steps, and sometimes the involved compounds inactivate or reduce the activity of biomolecules.

Direct immobilization of active biological substances in porous metal oxide carrier by physical entrapment *via* the sol–gel processes has drawn a great interest in recent years. This is due to its simplicity of preparation, low-temperature encapsulation, easy for immobilization, chemical inertness, tunable porosity, optical transparency, mechanical stability and negligible swelling behavior [26, 27].

The two major advantages with a sol–gel system is that it can retain a large content of water; this feature makes the encapsulated bio-recognition agents or enzyme catalytic centers long-term stable [28] and the process can be performed at room temperature. Other advantages of silica supports include biocompatibility and resistance to microbial attack. Moreover, the preparation conditions of a sol–gel have a remarkable effect on the activity of the entrapped active biomolecules [25].

The ability to form doped inorganic glasses under aqueous, room-temperature conditions (at which proteins and cells are active) opened up the possibility of extending sol–gel processing to the encapsulation of biomolecules. An array of substances, including catalytic antibodies, DNA, RNA, antigens, live bacterial, fungal, plant and animal cells, and whole protozoa, have been encapsulated in silica, metal-oxide, organosiloxane and hybrid sol–gel polymers [29]. The

sol–gel process as a route to form inorganic glasses has been known for over a century, however, the first report demonstrating the use of silicate materials for the entrapment of a biological moiety did not appear until the mid-1950s, when Dickey showed that several enzymes could be entrapped into silicic acid-derived glasses with partial retention of biological activity [30]. Unfortunately, the importance of this finding was not realized at the time, and the development of sol–gel derived biomaterials was not revisited for over three decades. Venton *et al.* showed in 1984 that antiprogesterone antibodies could be trapped within monolithic silica–poly(3-aminopropylsiloxane) sol–gel polymers and fully retain their native recognition and binding functions [31].

An year after, in 1985, Glad *et al.* showed that in monolithic and thick-film organic–inorganic sol–gel matrices comprising silica–poly[*N*,*N*-bis(29-hydroxyethyl)-3-aminopropylsiloxane] could be entrapped functional glucose oxidase, horseradish peroxidase, trypsin and alkaline phosphatase [32]. These studies allow Glad and coworkers to establish the critical features of sol–gel bioencapsulation: - the sol–gel technique permits the encapsulation of biomolecules; - the biocomposites display the characteristic activities of the entrapped species; - the polymer matrices are permeable enough to enable the diffusion of low molecular weight species, but not enough to permit the captured biologicals to leak. The studies of Glad group were recognized in 1990, when the technique was applied independently to the doping of transparent silica glasses with alkaline phosphatase, chitinase, aspartase and β-glucosidase.

In recent years, several reviews on sol–gel technology have appeared with specific applied areas [25, 26, 29, 33-39].

3.1. Sol-Gel Process and Bioencapsulation

As with traditional sol–gel fabrication, the starting point for bioencapsulation is the precursor (Fig. **6**). This is typically an alkyl silicate, an alkoxymetallate or an alkoxysilane, or a mixture of them [29, 40]. The precursor is hydrolyzed by water, either spontaneously or under acid or base catalysis, to form hydroxy derivatives (silicic acids, hydroxometallates, hydroxysilanes, etc.). A cascade of condensation reactions gives rise to soluble, colloidal and ultimately phase-separated polymers (polysilicates, hydrous metal oxides, polysiloxanes etc.), which produce the final matrices (silica, metallosilicate, metal oxide and siloxane) [29, 40]. Using this basic technique and specific fabrication processes (e.g. block casting, reverse emulsion polymerization, screen or contact printing, fluid-bed coating and dip- or spin-coating), one can obtain bio-doped hydrogels or xerogels in various configurations (e.g. monoliths, sheets, granulates, microparticles and thick and thin films) [29, 40].

Gill *et al.* [29] suggest in the review from 2000, the following model for the encapsulation of biomolecules:

- The biological entity resides within a polymer cavity whose interior is templated to conform physically and chemically to the surface features of the protein.
- There is substantial interpenetration of exposed biomolecule segments with the sol–gel polymer framework, resulting in varying degrees of embedding.
- A restricted solvent shell is trapped between the protein and polymer surfaces. Protein rotation and conformational transitions are restrained according to the conformity of the polymer surface, the composition of the sol–gel framework, the amount and mobility of the trapped solvent, and biomolecule – polymer interactions (ionic, hydrogen bonding, hydrophobic).
- There is sufficient accessibility between the binding or catalytic site of the protein and the pore structure, and enough freedom for local conformational transitions, to enable the entry, recognition and processing of substrates in a similar way to the free, soluble protein.

3.2. Types of Sol–Gel Precursor and Matrix

Much effort on bioencapsulation has focused on silica, metallosilicates and titanium, zirconium and aluminum oxides, which form hard transparent glasses that are micro- to mesoporous [40-43]. Since the first example of sol-gel bioencapsulation introduced by Avnir and co-workers [40], several types of sol-gel matrices have been developed to be used as substrates (Fig. **7**).

Inorganic Sol-Gels

The pure inorganic xerogels, such as aluminum, titanium, zirconium and tin oxides as well as their mixed oxides with silica, are always hard, transparent glasses with microporous structure. They are chemically robust, but limited by their brittleness and too small pore size, which prevents small molecule diffusion through the matrix [44].

Organically Modified Silica Sol-Gels (Ormosils)

In this category, organic groups, from simple alkyl, alkenyl, and aryl to those additionally bearing amino, amido, carboxy, hydroxy, thiol, and mixed functionalities as well as nicotinamides, flavins and quiniones, can be grafted on precursor silanes. Thus after sol-gel reactions, those organic functional groups are attached on the silica matrix by stable Si-C bonds. Because of those groups, tailorable properties, such as hydrophilic, hydrophobic, ionic as well as H-bonding capacities can be achieved in the silica matrix. However, the optical transparency and stability are lower than inorganic sol-gels [44].

Hybrid Sol-Gels

Amino- or hydroxyl- functional polymers, such as polymethyl silane, polyurethane, polyacrylate, and polyphosphazene are mixed with alkoxysilane during the sol-gel reaction. After polymerization, hybrid organic-inorganic structure can be formed in the silica matrix to provide good mechanical properties and variable hydrophilic-hydrophobic balances. But they are often not optically transparent and in some case only are available as hydrogels [45, 46].

Reinforced/Filled Composite Sol-Gels

To improve the mechanical properties and processing behavior of the sol-gel materials, some nano-or microparticles, such as graphite powder, fume silica, clays, cellulose and so on, can be incorporated inside the sol-gel silica. In addition, some active metal filler like gold, palladium,

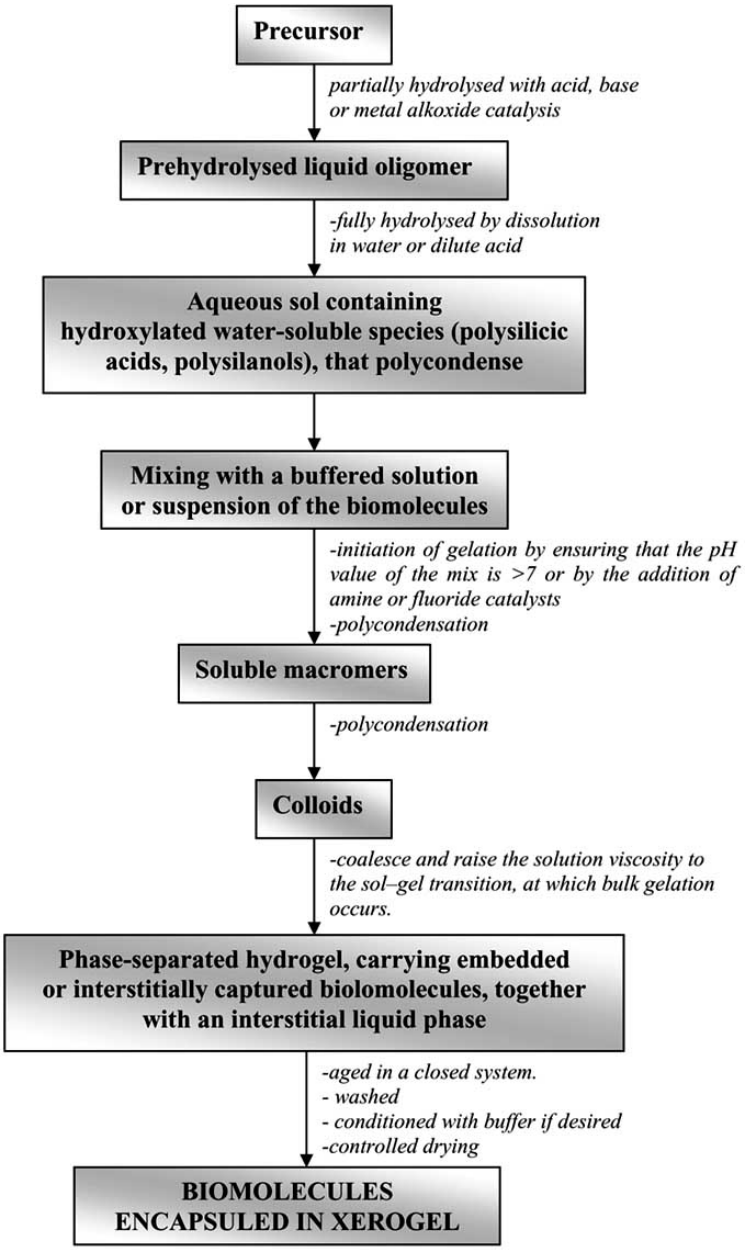


Fig. (6). The sol-gel bioencapsulation technique (adapted by Gill) [29].

platinum can be used when conducting and redox-active materials are desired [47, 48].

3.3. Enzyme (Biocatalysts) Immobilization

The excellent specificity and selectivity (including enantioselectivity) properties of enzymes have been employed to carry out processes of high complexity in less harmful experimental and environmental conditions. Among the immobilization methods described in the literature, bioencapsulation looks particularly valuable while direct linkage of the enzyme and support, frequently associated with activity loss, is avoided. The approach to sol-gel silica loaded with biological systems, particularly enzymes, has produced important and valuable implementations in biotechnology and open tremendous possibilities to understand the protein folding process [29, 34, 49, 50].

Silica has been widely used as an inert and stable matrix for enzyme immobilization owing to its high specific surface areas and controllable pore diameters, which can be tailored to the dimension of a specific enzyme: that is, microporous (<2 nm pore size), mesoporous (2–50 nm pore size) or macroporous (>50 nm pore size) silica. Because most enzymes are of the order of 3 to 6 nm in diameter, mesoporous materials are most commonly used [51]. One of the primary limitations of the sol-gel technique, however, is poor loading efficiency and enzyme leakage. The problem has in some instances been addressed by designing protocols for the preparation of matrices with a pore size that is adequate to allow the flow of substrates and products but small enough to prevent the elution of the entrapped biomolecules [52]. Recent interest in nanotechnology has provided a wealth of diverse nanoscaffolds that could potentially support enzyme

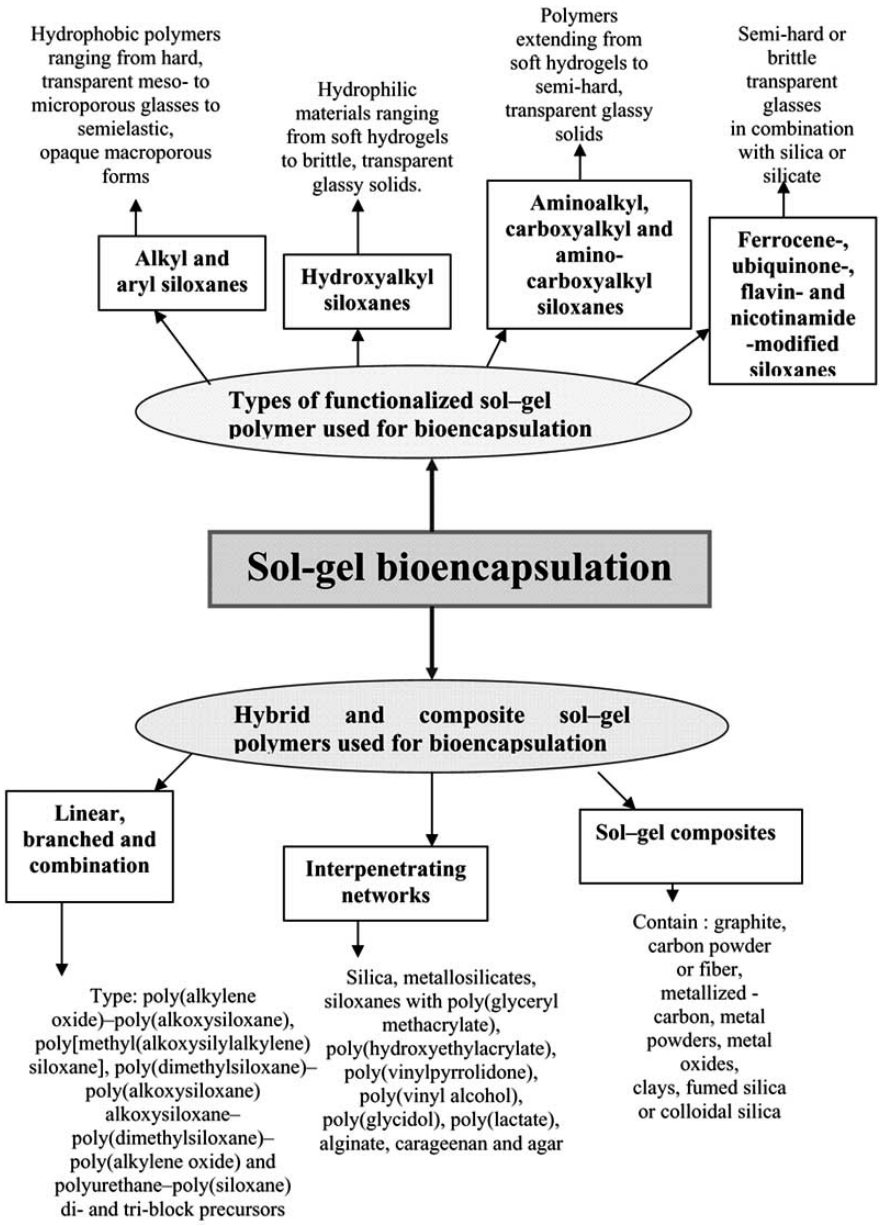


Fig. (7). Type of sol-gel precursors and matrix used in sol gel bioencapsulation (adapted by Avnir) [34].

immobilization and enzymes immobilized to nanosized scaffolds, such as spheres, fibers and tubes [53-55]. Betancor *et al.* [52] present in a recent review the advantages of biomimetic silica for enzyme immobilization: (i) *Inexpensive* (no need for chemical reagents for laborious synthesis); (ii) *Rapid* (immobilization occurs within seconds); (iii) *Mild conditions* (formation of the particles occurs at room temperature and neutral pH); (iv) *Nanosized* (Lower diffusion limitations and higher volumetric activities); (v) *Smart* (Matrix can be dissolved to release the entrapped enzyme); (vi) *Robust* (Physical properties suitable for flow-through applications); (vii) *Stabilizing* (Numerous enzymes have been stabilized by entrapment in this support); (viii) *Polymorphous* (Shapes can be tailored by varying the conditions of silica deposition).

Maintaining and improvement the enzyme characteristics as activity and enantioselectivity are the most important aim for every biocatalytic process. Selecting the appropriate precursors, additives and template compounds is fundamental to obtain solid-phase biocatalysts with higher efficiency and operational stability. Ionic liquids could have an important function in this issue, as they contribute to the formation of the sol-gel framework and can also facilitate the enzymatic reaction by their hydrophilic or hydrophobic nature. The results provide that fine tuning of ionic liquid and silane precursor structure can result in entrapped lipase composites with higher activity and enantioselectivity than the native enzyme. However, several other aspects are yet to be improved, as avoiding sol-gel matrix shrinkage and enzyme aggregation during the immobilization process [56].

Enzyme interactions with the sol-gel matrices lead to conformational changes of the lid that result in an open, substrate-accessible form of the enzyme, and to the formation of the oxyanion hole. This phenomenon is found with most

lipases and also with cutinase. Cutinase is a versatile enzyme that catalyzes synthetic and hydrolytic reactions on a wide range of substrates in aqueous and non-aqueous media [57]. Cutinase from *Fusarium solani pisi* was encapsulated in sol–gel matrices prepared with a combination of alkyl-alkoxysilane precursors of different chain-lengths.

Another example refers to sol–gel encapsulated creatine kinase that is protected from thermal denaturation. It retains 50% of its activity ten times longer at 47^{o} C (125 hours) than the free enzyme (13 hours) at the same temperature. Surprisingly, a 4-fold increase in activity is even observed upon heating for a short time. This was explained by structural changes in both enzyme and the sol–gel matrix. Actually the structure of the creatine kinase is slightly modified upon encapsulation, but reverts to the right conformation upon heating. Moreover, short heating could also increase the pore size of the silica matrix allowing easier diffusion and decreasing the stresses arising from encapsulation [58].

Tripsin inhibitor was chosen as the model protein, because its size (21 kD) is similar to that of bone growth factors. The viability of using sol-gels intended to serve as both substrates for bone growth as well as to allow incorporated proteins such as growth factors to diffuse out and stimulate cell function and tissue healing was under investigation. The data documented that the *in vitro* release of tripsin inhibitor was dose and time dependent during immersion up to 9 weeks [59].

Sugar (sorbitol) and amino acid N-methylglycine additives can be used to stabilize enzymes within sol–gel matrices. Chymotrypsin and ribonuclease T1 have been trapped in the presence of sorbitol and N-methylglycine. Both osmolytes significantly increase the thermal stability and biological activity of the proteins by altering their hydration and increasing the pore size of the silica matrix [58, 60].

D-glucolactone and D-maltolactone have also been covalently bound *via* a coupling reagent (APTS) to the silica network giving non hydrolyzable sugar moieties. Firefly Luciferase, trapped in such matrices, has been used for the ultra sensitive detection of ATP *via* bioluminescent reactions [58].

Bioconversion Using Sol–Gel Entrapped Enzymes

The enzymatic methods are suitable for the synthesis of optically active compounds. Often only one enantiomer of a compound has a pharmacological effect, whereas the other enantiomere has no, or an unwelcome, effect. Therefore, biotechnical syntheses gain importance in the production of compounds requiring high enantiomeric purity for pharmaceuticals or commercially important products [25].

Reetz *et al.* established the practical utility of sol–gel immobilized biocatalysts with the encapsulation of lipases in Ormosils [61]. They reported a technique of sol gel bioencapsulation which induce lid-displacement activation of lipases during sol–gel formation and leads to the surface encapsulation of the activated lipases in micro-phase separated poly(alkylsiloxanes). Their method appears to be generic for lipases and produces highly active particulate and thick-film immobilization, which mediate regio-, chemo- and enantioselective transformations in aqueous–organic media and organic solvents [61, 62]. The promise of the technique is indicated by the high immobilization efficiencies, indicated by the high immobilization efficiencies, increased resistance to denaturation and enhanced storage and operating stabilities of hydrolases, oxidoreductases, lyases and transferases entrapped in silica, metallosilicate, Ormosil and composite sol–gels [29, 61, 62].

One of the most powerful applications of sol–gel biocatalysts is the co-entrapment of multi-enzyme systems, in which the nanoconfinement of the catalysts and reactions increases the effective concentrations of reaction intermediates, thereby enhancing overall efficiencies. Thus, sialic-acid aldolase, myokinase, pyruvate kinase, pyrophosphatase, CMP–sialate synthase and (2,6)-sialyl transferase can be trapped in poly(3-aminopropylsiloxane)-zirconosilicate to enable the continuous synthesis of a(2,6)-sialyl-*N*-acetyllactosamine [29]. Also, formate dehydrogenase, formaldehyde dehydrogenase and alcohol dehydrogenase have been co-encapsulated in silica to allow the high-yield conversion of carbon dioxide to methanol [63].

By co-immobilizing formate dehydrogenase, formaldehyde dehydrogenase and alcohol dehydrogenase in silica gels, together with the electron donor NADH (Nicotinamide adenine dinucleotide), synthesis of methanol can be performed at low temperatures and pressures [64, 65].

Pierre [66] in his review of the sol–gel encapsulated enzymes and their applications concluded that even though the sol–gel entrapped enzymes show improved stabilities against physicochemical changes around their vicinity and activity; still, they are facing diffusion limitations, which lead to the high *K*m value and have great impact on conversion at large scale use of immobilized enzymes in industrial applications. Hence more efforts are needed for the improved diffusion properties of sol–gel matrices for efficient biotransformation/synthetic reactions.

3.4. Antibodies Immobilization

The antibodies encapsulated in sol–gel-derived glasses can interact with target molecules with a high degree of specificity as in solution, and the signal can be detect using an appropriate sensing scheme. Generally antibodies are high molecular weight proteins. If antigen is also high molecular weight compound the interactions between antibody and antigen is difficult through the small pores of the matrix [67]. In such a case, the antigen can be tagged with small signaling compounds such as ferrocene or their derivatives, depending upon the detection mode [68]. Wang *et al.* [69] encapsulated firstly antifluorescein antibodies in TMOS sol–gel. Yang *et al.* [70] reported that the addition of poly(ethylene glycol)-PEG did not affect the encapsulation efficiencies of the antigentamicin antibody but determined a strong effect on the binding activity of the encapsulated antibody . When the antibody was encapsulated in sol–gel along with PEG, 95% of the gentamicin was bound to the column comparative with the case of sol gel without PEG when only 42% was bounded to the column. Other studies also reported that the addition of PEG stabilized the urease and prevented the fouling and adhesion of unwanted protein to the surface [71].

Shabat *et al.* [72] successfully encapsulated catalytic antibodies through sol–gel method and used them in the trans-

formation reactions. Antibody 14D9 is an effective catalyst for various hydrolytic reactions including the hydrolysis of a cyclic acetal, ketals, epoxides and enol-ethers. The catalyst has been homogeneously doped inside the gel matrix and shows a catalysis followed Michaelis–Menten kinetics. The entrapped antibody is more stable than that immobilized through surface attachment [25]. The antigentamicin Mab entrapped in mesoporous TMOS so-gel monolith was employed for the development of flow injection fluorescence immunoassay for the quantitative analysis of the gentamicin [69].

The development of the novel hepatitis B surface antigen (HBsAg) immumosensor was achieved by self-assembling gold nanoparticles to a thiol containing sol–gel network. Thus, a gold electrode was first derivatized with mercapto-propyl-trimethoxysilane sol–gel solution, forming a mercapto-silica gel layer, and then gold nanoparticles were chemisorbed onto the thiol groups. Finally, hepatitis B surface antibodies (HBsAb) were adsorbed onto the surface of the gold nanoparticles. The electrochemical ferricyanide redox process was used as a probe to determinate HBsAg. It was found that this approach is superior to the glutaraldehyde binding approach, in terms of the larger amount of adsorbed antibodies immobilized by this method and in the terms of higher immunoactivity [73].

Liu *et al*. [74] prepared luminescent sol–gel based silica nano-particles (20 nm), doped with dibromofluorescein (D–SiO_2), placed on a polyamide membrane. The particles phosphoresce intensively and the phosphorescence can be quenched with $Pb(Ac)_2$. This system was used to detect human IgG, through the interaction with a goat-anti-human IgG antibody labeled with D–SiO_2, by following quantitatively the regained phosphorescence intensity of the particles due to the immunoreaction. The limit of detection is quite low (0.018 pg per spot), and the reproducibility is very good, with about 4% deviation over 11 measurements.

3.5. Protein Immobilization

The major advantages of sol–gel derived silicate materials for immobilisation of proteins are [26] : (i) they can be made to be optically transparent, making them ideal for the development of chemical and biochemical sensors that rely on changes in an absorbance or fluorescence signal, (ii) they are open to a wide variety of chemical modifications based on the inclusion of various polymer additives, redox modifiers and organically modified silanes (Ormosils) (iii) they have a tunable pore size and pore distribution, which allows small molecules and ions to diffuse into the matrix while large biomolecules remain trapped in the pores, allowing size-dependent bioanalysis.

Braun and co-workers [33] described the entrapment of proteins into alkoxysilane-derived silicate materials using the sol-gel method. Two years after, Dunn's and Zink's groups [75], demonstrated that other proteins, such as cytochrome *c* and myoglobin, could be entrapped into TMOS derived silicates with retention of O_2 binding ability [63, 76].

Entrapped proteins typically reside in pores that are of a similar size to the protein, thus, it is important to ascertain whether entrapped proteins maintain their native conformation during and after entrapment, and whether they are able to undergo changes in conformation once entrapped. The latter point is particularly important since in many cases the binding of an analyte to a protein requires that the protein be able to undergo conformational changes, which may in turn be used to derive an analyte-dependent fluorescent signal, as is the case for a number of fluorescent allosteric signal transduction proteins [26, 77].

Conformational studies of a variety of entrapped proteins [monellin, parvalbumin, oncomodulin, human serum albumin (HSA) and bovine serum albumin (BSA)] have indicated that such proteins tend to retain a native conformation immediately upon entrapment, although some proteins, such as myoglobin, may undergo substantial conformational changes during entrapment [78].

Another aspect of entrapped proteins that has been widely studied is the conformational stability of the protein in the presence of denaturing stresses. These studies have indicated that the conformational motions of large proteins, such as BSA, can be substantially restricted in the sol–gel media and in some cases, inactive conformations may end up "trapped" in the sol–gel matrix [79, 80].

Overall, studies of conformational motions of entrapped proteins suggest that the conformational stability of the native form of a protein determines to a large degree the conformational changes that are induced by sol–gel entrapment and the degree of stabilization that is imparted upon entrapment. That is "hard" proteins (such as cytochrome c and antibodies, which are rigid and do not easily undergo conformational changes more easily) tend to maintain their native conformation in the glass, while on the other hand, "soft" proteins (such as HSA or BSA, which are more flexible and undergo conformational change more easily) tend to denature. Furthermore, such studies indicate that large scale motions (e.g. folding and unfolding) in the sol–gel is greatly restricted, especially in dry-aged gels, but segmental motions (including those required for substrate binding) are largely unaffected.

Various electrochemical and spectroscopic studies on a number of proteins in silica hydrogels and xerogels showed the existence of native conformation of protein along with restricted rotations and global conformational changes inside tight silica cages and still the possibility of local motions required for binding and catalysis [29] These studies showed unambiguously that large-scale dynamics of biomolecules is strongly hindered in the glassy cage. Various physical forces, e.g., specific electrostatic interactions between silicate sites and protein surface residues and mechanical forces have been implicated to reduce flexibility of the entrapped protein.

The most important issue regarding entrapped proteins is whether they remain functional, and to what degree. The function of an entrapped protein depends on a number of factors, including the protein location (related to accessibility of the protein, as described above), protein structure (native versus unfolded), and the charge and polarity of the local environment, which can affect the binding properties of the protein [26].

4. THE APPLICATION OF SOL-GEL TECHNOLOGY IN DRUG DELIVERY

The research on sol-gel technique has been made to have application in different fields and recently have strong attention for controlled drug-delivery. Porous materials, xerogels, organic-inorganic hybrids and nanocomposites can be elements for pharmaceutics studies.

The synthesis of controlled release sol-gel system involves several steps: an acid-catalyzed hydrolysis to form a sol with the bioactive molecules included, followed by casting, aging and drying. To produce porous sol-gel particles with desirable size there are requested additional steps, like grinding or sieving. Other important parameters of synthesis are pH and time of gelation, drug concentration in the sol, the rotational speed and time of drug release system [81]. The drugs are chemically (by covalent bonding) or physically (by adsorption) incorporated into the sol-gel system.

The therapeutically agents are selected, depending on the application. The more required drugs to be included in a sol-gel system embrace: anti-thrombotic agents, anti-proliferative agents, anti-inflammatory agents, anti-migratory agents, anesthetic agents, anti-coagulants, cell growth promoters, cell growth inhibitors and combinations thereof. Depending on the genetic or non-genetic action, the therapeutically agents are classified into two classes.

In the Table **1** are summarized the types of non-genetic therapeutic agents.

The genetic therapeutic agents are in principal: i) antisense RNA; ii) tRNA or rRNA used to replace defective or deficient endogenous molecules; iii) angiogenic and other factors like: acidic and basic fibroblast growth factors, epidermal growth factor, transforming growth factor α and β, platelet-derived endothelial growth factor, platelet-derived growth factor, tumor necrosis factor, hepatocyte growth factor, insulin-like growth factor; and iv) cell cycle inhibitors.

By using targeted drug-delivery systems based on polymers, liposome or microsphere, the toxic effects of certain drug types can be mastered without decreasing the drug potency. In this regard, there is produced the encapsulation of doxorubicin in a polysiloxane by sol-gel method and the good results are correlated with the short time of gelation and high efficiency of the drug encapsulation, which is realized by pre-doping method, but with low drug release. The pre-doping method refers to the fact that the drug loading was done with the gel formation [81].

The drug encapsulations in the hybrid organic-inorganic matrices are influenced by few processing parameters, such as porosity degree and specific surface of the pores, increasing of the organic part in the synthesis, aging/drying conditions [81]. After some tests, there is observed the release of drug it is lowered by adding organic precursors..

The drug-release behavior can be explained by considering the effect of textural properties of the xerogels (matrix swelling, matrix dissolution), the bioavailability and solubility of the drug in aqueous media, pH , temperature, type or

Table 1. The Non-Genetic Therapeutic Agents

Class: non-genetic therapeutic agents	
1. Anti-thrombotic agents	heparin, synthetic heparin analogs, urokinase, dextrophenylalanine, proline, arginine;
2. Antibiotics	vancomycin, doxorubicin, cefoxitin, tetracyclines, chloramphenicol, neomycin, gramicidin, kanamycin, amikacin, sismicin
3. Anti-inflammatory steroids agents (SAIDs)	cortisone, hydrocortisone, estrogen, dexamethasone, fluocortolone, prednisone, triamcinolone, budesonide, sulfasalazine, mesalamine, fluocinolone
4. Non-steroidal anti-inflammatory drugs (NSAIDs)	flurbiprofen, ibuprofen, indomethacin, piroxicam, naproxen, antipyrine, phenylbutazone, aspirin, diclofenac, fenoprofen, ketoprofen, mefenamic acid
4. Antineoplastic /antiproliferative / antimiotic agents	paclitaxel, 5-fluorouracil, cisplatin, vinblastine, vincristine, epothilones, endostatin, angiostatin, angiopeptin
5. Anesthetic agents	lidocaine, bupivacaine, ropivacaine, procaine, benzocaine, xylocaine
6. Anti-coagulants	heparin, hirudin, antithrombin compounds, platelet receptor antagonists, anti-thrombin antibodies, anti-platelet receptor antibodies, aspirin, prostaglandin inhibitors, platelet inhibitors
7. Vascular cell growth promoters	growth factors, transcriptional activators, translational promotors
8. Vascular cell growth inhibitors	growth factor inhibitors or receptor antagonists, transcriptional or translational repressors, replication inhibitors, inhibitory antibodies, antibodies directed against growth factors
9. Protein kinase and tyrosine kinase inhibitors	tyrphostins, genistein, quinoxalines
10. Antimicrobial agents	triclosan, thimerosal, chloramine, boric acid, phenol, cephalosporins, aminoglycosides, nitrofurantoin;
11. cytotoxic agents, cytostatic agents, cell proliferation affectors	

concentration of the drug, the addition of acids, aging and drying times [82].

However, although the release of bioactive agents may be delayed, the release phenomena from sol-gel systems may exhibit large fluctuations, which can lead to unfortunate side effects. This shortcoming can be adjusted using water–soluble substance to accelerate the release rate of drug or modifying agents that increase the permeability of the matrix by including polyanionic compounds in the synthesis.

Three types of interaction between drugs and sol-gel system can affect the release rate: electrostatic interaction, hydrogen bonding and hydrophobic interaction. The strength of these interactions is given by the functional groups of the main component of the system and the nature of dissolution medium [82]. Function of these interactions the release of the therapeutic agent from a sol-gel system can be achieved by several mechanisms: diffusion, osmosis, ion exchange or by degradation of the system components. For a matrix system obtained by sol-gel technique, in almost all cases the release kinetics obeys to Higuchi model [83]. The Higuchi model is used both for water-soluble and low soluble drugs incorporated in solid/semi-solid matrix. If the matrix includes biodegradable polymers in biological medium, governed by the local enzymes, the drug release may occur by degradation mechanism [84]. When the drug is released due to osmotic swelling, the kinetics release fit to Fick´s law. In this case the water penetrates into the drug carrier system, inducing relaxation of polymer chain and thus, the drug is released outside.

The influence of the components' ratio on the drug release properties of poly methylmethacrylate/3-(trimethoxysilyl) propyl methacrylate/silica composite has been evaluated using aspirin as a model drug. Mei *et al*. [85], conclude that the drug release fitted with the Fickian diffusion model and is influenced by the coupling agent content and the interface between the polymer matrix and the silica. Thereby, with the increase of the coupling agent content, 3-(trimethoxysilyl) propyl methacrylate, it can obtain a low degree of the released drug *in vivo* and it can reduce the toxic levels, into the body, due to a sudden drug release.

The sol-gel products have a promising future due to the successful results in the medical field. The sol-gel technology was accepted and considered safe for human use. The U.S. Food and Drug Administration have acknowledged receipt for a product based on therapeutic agent encapsulating in silica microcapsule to treat rosacea [86].

In the following some examples concerning the different geometry and applications of drug delivery systems obtained by sol-gel method, are presented.

4.1. Drug Delivery from Nanoparticles Synthesized by Sol-Gel Method

The most candidates for controlled drug-delivery particles synthesized *via* sol gel route are derived silica particles, which have the capacity to encapsulate biologically and therapeutically active molecules and release to the application. The sol-gel derived silica particles show a very slow degree both for drug loading and release of adsorbed bioactive agents than the soft silica gels [87].

A series of nanoparticles based on organic-inorganic materials synthesized by co-hydrolysis and co-polycondensation reactions, led to the formation of a core–shell structure with a hybrid core of silica-polymer containing hydroxyl groups and a silica-polymer containing poly(ethylene-glycol) groups monolayer shell. The hydroxyl methyl triethoxy silane and ω-methoxy (polyethyleneoxy) propyl trimethoxy silane can form by sol-gel method, water-soluble organo-silica particles with controllable size and molecular weight. These organico-silica nanoparticles are expected to be useful in drug delivery application [88]. The efficiency of drug delivery is directly affected by particle size, the geometric shape of the particles, the main compounds, the natural/synthetic type of the polymer, etc.

The biodegradability of silica particles investigated, *in vitro*, in simulated physiological buffer, shows a linear behavior for the dissolution rate. This aspect is important when the drug-silica system is used for an implantable therapeutic system. When added the serum proteins in the test solution, the degradation rate was slower by 20-30% [89, 90].

4.2. Drug Delivery from Porous Systems Synthesized by Sol-Gel Method

An advantage in drug release phenomena is to have a porous morphological aspect, obtained by controlled drying or by using a modified catalyst molar ratio during the synthesis [91]. The pore surface is significant because it serves at the absorption for different types of molecules, such as bioactive agents, enzymes, proteins and growth factors. In addition, such drug carriers improve the therapeutic efficiency, change pharmacokinetics and provide protection from degradation, which is necessary in certain circumstances. The influence of synthesis parameters were studied upon different sol-gel / drugs matrix.

Few general methods relying for drug incorporation in the matrix can been considered: drug incubation, drug *in situ* incorporation [92], by combining sol-gel polymerization with spray-drying or emulsion chemistry [93], spray-drying. The drug incorporation after the porous sol-gel system was synthesized has the advantage to avoid the permanent bonding of drug into organic-inorganic system [94].

Nanostructured amorphous microporous silica, synthesized under acid-catalyzed sol-gel conditions, represents a support for drug incorporation with oral controlled release. To confirm these assumptions, ibuprofen it was dispersed in the silica matrix and the release profile was viewed in a medium simulating of gastrointestinal tract buffer. It is concluded that the drug release rate can be tailored by adjusting the pore diameter [95].

A porous matrix as a host for magnetic nanoparticles has attractive application in drug delivery. The magnetic particles with unique properties can bind and transport drugs to their structure. The advantage of including nanoparticles in silica matrices is that it can protect the metallic particles against air-oxidation. Usually the particles are embedded inside the pores by in situ sol-gel synthesis or by impregnation of mesoporous silica gels [96].

4.3. Drug Delivery from Gels by Sol-Gel Method for Orthopedic Application

Ambrosio *et al.* [97] were synthesized hybrid organic-inorganic amorphous materials to control the release mechanism in simulate body fluid for a broad-spectrum antibiotic. The hybrid materials based on silica/polycaprolactone form a wet gel with sodium ampicillin by sol-gel method. This system was designed to treat bone infection in orthopedic surgery and have a good rate of bone integration and regeneration [97].

4.4. Pro-Drug Delivery by Sol-Gel Method

The enzyme L-amino acid decarboxylase is incorporated into a biocompatible silica-organic carrier sol-gel matrix. This complex is transferred in the brain, where converts L-dopa to dopamine and help in treatment of Parkinson's disease. The pro-drug may be formulated for administration by, for example, injection, inhalation or insufflations (either through the mouth or the nose) or oral, buccal, parenteral, rectal administration or implanted subcutaneous form [98].

4.5. Molecular Imprinting of Drugs as Drug Delivery Systems

Molecular imprinting is an emerging field that produces precise chemical architecture that can bind analytes and differentiate between similar molecules with enantiomeric resolution. The recent growth in interest in organic–inorganic hybrid materials prepared by sol-gel chemistry and the development of a variety of new strategies for imprinting polymeric matrices have led to a growing activity in what became known as molecularly imprinted sol-gel materials [99]. By combining sol-gel process with molecular imprinting it can create ideal drug carriers with different medical application fields [100]. The indomethacin, naproxen and ketoprofen as model drugs, were incorporated into silica derivate system by molecular imprinting technique. The results show the selectivity for type of drug bound in the system to the functional silane and the specific geometric cavity.

4.6. Drug Delivery from Thin Films Obtained from Sol-Gel Method

A thin film, prepared under acid-catalysis through a sol-gel method and tetraethyl orthosilicate precursor synthesized for coating Ti-6Al-4V wires, shows remarkable properties to prevent and treat the bone infection. The thin film acts as antibacterial film when vancomycin-antibiotic is incorporated [101].

To improve the sol-gel barrier films, a tetrafunctional alkoxide silicate and Lewis acid or metal chelate like catalyst were investigated to form a crosslinked sol-gel polymer composition, where the polymer composition is uniformly coated on a substrate and dried. The application of this kind of research is for oxygen barrier films [102].

The thickness of the sol-gel films is capable to adjust the drug release rate by diffusion mechanism [103]. Organic – inorganic hybrids can be used to cover the compressed tablets which include therapeutic agents. For example a polydimethylsiloxane-sol-gel silica hybrid was tested for coating of tablets with hydrochlorothiazide [90].

4.7. Drug Delivery by Sol-Gel Method for Tissue Engineering Applications

Great attention should be given to biomaterials used like wound dressings, because they must include both growth factors and anti-inflammatory drug. Once applied, the wound dressing remains in the body where it gradually degrades.

The scaffolds should provide space and substrate for barrier against bacteria, cell differentiation and proliferation. The dextran and Vancomycin in a sol-gel/copolymer composite was synthesized such as a film for wound dressing. Tyrosine-polyethylene glycol (Mw = 1000 Da)-derived polycarbonate composite with silica xerogel shows good mechanical properties and have the ability to bind and release gradually bioactive molecules [104].

4.8. The Application of Sol-Gel Technique to Achieve Medical Devices

Other application of sol-gel technique is to realize medical diagnostic tools, which require biocompatibility, non-toxicity, bioactivity and/or drug delivery properties. The medical devices must be without inflammatory tissue responses or immune reactions, if they are used like implant materials such as surgical and/or orthopedic devices, artificial heart-valves, and stents covered with polymeric thin film.

This type of medical apparatus is accompanied by selective sensing elements in the form of thin film, porous structures, which are sensible systems in non-invasive monitoring and different diagnosis of diseases [105]. Polymeric part can hold the drug onto the surface of implantable medical devices, and controlled drug release occurs *via* degradation of the polymer or diffusion into liquid or living tissue [106].

Some implantable or insertable medical devices comprising a sol-gel derived ceramic region with a porous structure and different geometries were realized. Ceramic component was molded to comprise metal oxides, semi-metal oxide and polymers. The results showed that the use of polymers as substrates improves the properties of devices. The preferred polymers were selected, such as polymethylmethacrylate, polytetrafluoroethylene, various polyvinyl polymers and polyurethanes. A polymer can be introduced to the sol-gel derived ceramic by infiltrating the polymer into the pores of the template or the monomer may be polymerized in the presence of the template. For a polymer in fluid form, it may be introduced to the template by spin coating, spray coating, dip coating, ink jet printing, coating with an applicator such as a roller brush or blade and solidified. The ceramic region corresponds to the medical device in its entirety or to a discrete component of the medical device and can incorporate a therapeutic agent. These implantable or insertable medical devices are susceptible for non-planar, tubular medical device and stent [107].

Several studies and Patent Applications describe the use of sol-gel compositions as drug reservoirs on implantable medical devices and to improve adhesion between organic and inorganic surfaces, too.

Examples for suitable implants are tooth-implants, hip-implants, knee-implants, mini plates, external fixation pins,

stents (e.g. for use in repair of blood vessels) or any other metallic, polymeric, ceramic or organic implants that can be coated with a layer of sols and gels, and/or sol-gel derived materials, the therapeutic agent being incorporated into this coating. The coating dissolves in the tissue and releases the active substance locally [108]. Titanium, alloys of nickel and titanium (NiTi-alloys), other memory-shape metals, Al_2O_3 or other ceramic materials can be covered with organic thin films to increase the properties, such as biocompatibility.

Promising for the future medical devices are those covered with an organosilane thin film obtained by sol-gel technique. Such equipments are resistant to oxidative or corrosive medium, especially resistant to iodine [109]. The coating process can be obtained by partly hydrolytically condensing an organosilane compound, thereby forming a sol, which sol is subsequently provided on a substrate. After the hydrolytic condensation is finished by curing at an elevated temperature, it is formed a network with drug loading possibility.

5. SOL-GEL TECHNIQUE FOR BIOSENSOR PREPARATION

The organic–inorganic sol–gels have also received significant interest because the incorporation of organic polymers in the inorganic sol–gel can lead to new composite materials possessing the properties of each component that would be useful in particular applications. Incorporation of organic polymers, especially those with amino or amide groups, allows the formation of molecular hybrids often stabilized by strong hydrogen bonding. Thus, the sol–gel technique provides a unique method to prepare the three-dimensional network suited for the encapsulation of a variety of biomolecules – enzymes or proteins – as was already mentioned as well as sensors [110-112].

The incorporation of sensing molecules in suitable matrix and monitoring/quantitating the interactions between the analytes and these molecules represent the principal tasks for performing suitable biosensors. Many biological macromolecules are highly efficient at recognizing specific analytes or catalyzing reactions in aqueous biological media. These characteristics make biomolecules desirable reagents [113]. Immobilization of biomolecules has become the most important area of research in the development of biosensor. Various immobilization techniques have been applied, including adsorption to solid supports, covalent attachment, tethering *via* an intermediate linker molecule and entrapment in polymers (Fig. **8**) [3].

For the electrochemical biosensor, the effective immobilization of an enzyme on an electrode surface with high retention of its biological activity is also a crucial point [114-116]. Among various approaches, the sol–gel method of processing is particularly advantageous to the immobilization of biomolecules [117].

Immobilization of enzymes with low temperature sol–gel process became a topic of ongoing research owing to its advantages as well as to the porous and open frame works in the sol–gel silica materials which provide effective access of the analyte into the active functionalities and could result in high sensitivity for the detection of analyte. The various sol–gel systems such as silica, alumina, and titania have been reported to be suitable materials for immobilizing glucose oxidase (GOD), horseradish peroxidase (HRP), tyrosinase and other biomolecules [117-123]. Braun *et al*. firstly reported the attempt to encapsulate proteins inside SiO_2 glasses in 1990 [33]. Since then, these new kinds of inorganic materials are particularly attractive for the development of electrochemical biosensor [123].

However, besides complicated procedures and fragility, the shrinkage and cracks of the sol–gel silicate modified membranes greatly decreased the stability of the biosensors and limited their applications. Other non-silica sol–gel matrices have been developed to immobilize enzymes for fabrication of the biosensors. Deng and co-workers [118] immobilized tyrosinase in a positively charged Al_2O_3 sol–gel matrix instead of negatively charged silicate matrix, which was more suitable for negatively charged tyrosinase in neutral solution [124].

Another alternative substrate for biomolecule immobilization is nanocrystalline TiO_2. Durrant and co-workers investigated the use of nanoporous TiO_2 film as substrates for protein adsorption. They proved that the nanoporous structure of the titania film greatly enhanced the active surface area available for protein bonding. In another paper, they compared the use of nano-TiO_2 and ZnO as substrates for protein immobilization and as electrode probes for electrochemical reduction of the absorbed substrates [125, 126]. Wang *et al*. [126] illustrated that titania sol–gel carbon composite electrodes could offer a substantial decrease in the over voltage required for nicotinamide adenine dinucleotide (NADH) oxidation, as well as minimization of surface-fouling effects [127].

As it was mentioned before, the enzymes and proteins immobilized within sol–gel matrices maintain their native properties and reactivates, which assure the development for biosensors. Also, the sufficient amount of trapped interstitial water in gels allows the retention of the tertiary structure and active reactivity of encapsulated biomolecules. At the same time, the pore size in sol–gel can be controlled into an appropriate size for the diffusion of the analyte to the redox active sites, as well as preventing enzyme leakage. Even so, some papers mention that some gels lose their activities after the first few cycles, primarily ascribed to leaching of enzymes from the sol–gel matrix [128].

5.1. Sol–Gel Organic Hybrid Composite Biosensor

As a consequence, a suitable method for immobilization/entrapment is still a challenge in the development of the biosensor field. Sol–gel glass offers a better way to immobilize biomolecules within its porous optically transparent matrix and demonstrated functional activity of encapsulated biomolecules. This is due to simple sol–gel processing conditions and possibility of tailoring for specific requirements. Due to this inherent versatility, sol–gel-derived glasses are excellent host matrix for chemical sensing and biosensing. This approach is unique compared to the conventional methods involving adsorption on glass surfaces, entrapment in polymer matrices or impregnation in porous glass powders because entrapment is based on the growing of siloxane

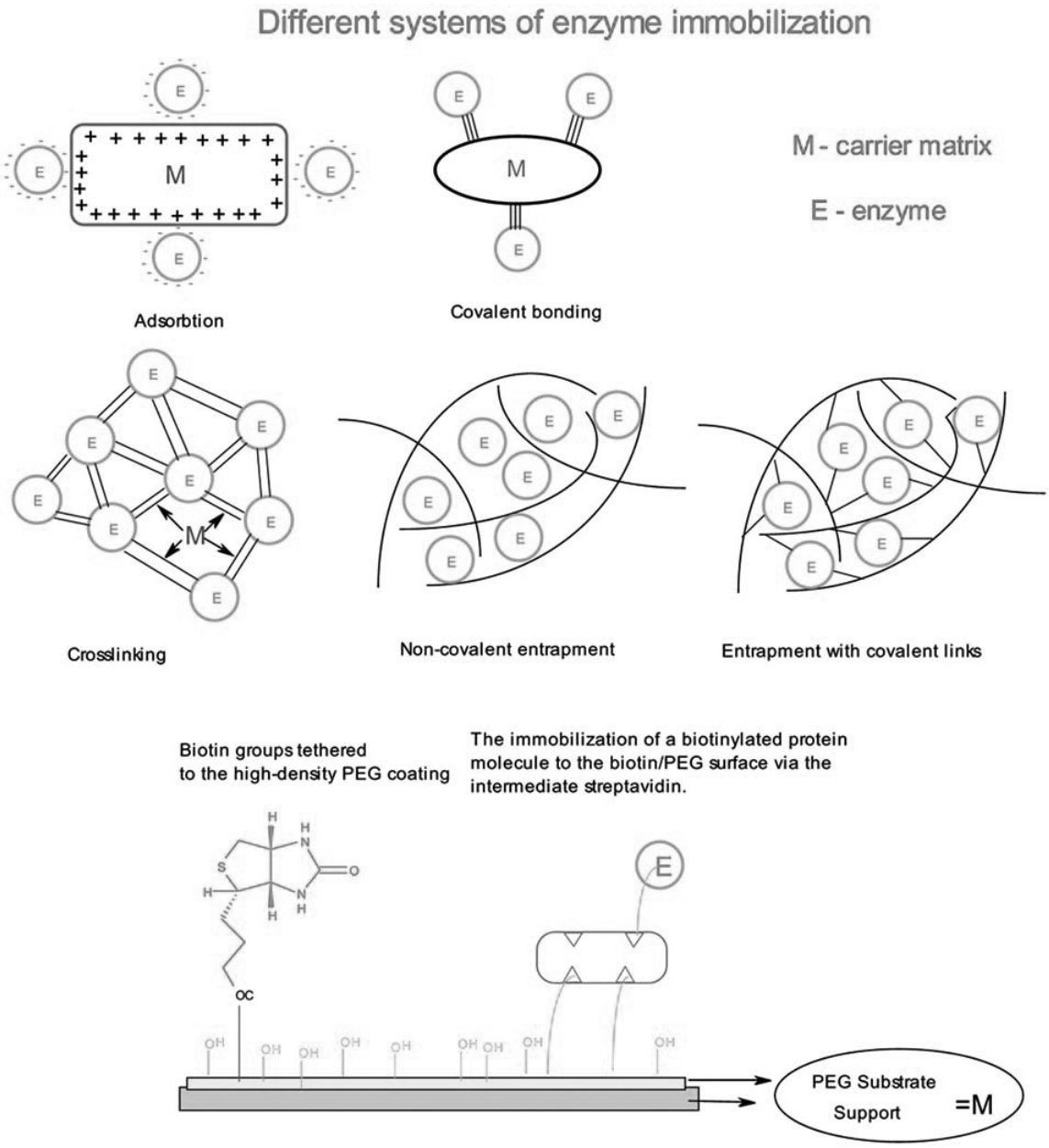


Fig. (8). Immobilization techniques: adsorption to solid supports, covalent attachment, tethering *via* an intermediate linker molecule and entrapment in polymer networks.

polymer chains around the biomolecule within an inorganic oxide network. Also, because of the porous nature of the sol–gel network, entrapped species remains accessible and can interact with other external chemical species or analytes. At the same time, sol–gel-based sensors suffer from some disadvantages, e.g., entrapment in sol–gel glass may change chemical and biological properties of the entrapped species, due to reduced degrees of freedom and interactions with the inner surface of the pores. The entrapping environment of the biomolecules must be careful chosen since this will affect both stability and functionality of the sensor. The analytes must also have the capacity to enter the glass by diffusion to the site of entrapped biomolecules so that measurable signals to be generated from the interaction process for sensing. Research studies present and underline the effect of various physical-chemical properties, e.g., pH, polarity and microviscosity of the environment within sol–gel-derived matrices as well as the structure, dynamics, activity and function of the biomolecules [128-132]. Some reports also, focused on the effect of long-term sol–gel matrix aging on dynamics of protein and the detailed kinetics of the interaction between analytes and entrapped proteins. Occurrence of appropriate environment around entrapped biomolecules and its long-term stability is one of the important factors determining the functionality of entrapped proteins and enzymes [133-135]. The characterization of the internal environment of the sol–gel matrix is one of the focuses of research activities to design appropriate matrix for sensing applications. Thus, it have been made attempts to characterize the internal environment of the bulk gel and the thin gel films prepared

from different sol compositions using various fluorescent molecules as a function of long-term storage. Fluorescence spectroscopy was used in this order.

A fundamental component of amperometric biosensors and biofuel cells are enzyme-modified electrodes. The selection of appropriate combinations of materials, such as: enzyme, electron transport mediator, binding and encapsulation materials, conductive support matrix and solid support, for construction of enzyme-modified electrodes also governs the efficiency of the electrodes in terms of electron transfer kinetics, mass transport, stability, and reproducibility [136].

The inorganic M–O–M (M-metal, O-organic) or M–OH–M bridges into the sol–gel materials form a continuous network able for bioimmobilization as well as includes a liquid phase which can then be dried out to form a solid, porous polymeric matrix. In biosensors, this technique allowed the development of novel strategies for the immobilization of biological receptors within silica, metal oxide, organosiloxane, and hybrid sol–gel polymers, which finally induces advanced materials [137].

Silica sol–gel material prepared under ambient conditions is well biocompatible and can retain the catalytic activities of enzymes to a large extent. Thus, it has emerged as one matrix well suited for the immobilization of enzyme, the construction of hydrogen peroxide biosensor.

Lim *et al*. fabricated nanostructured electrodes for enzymatic glucose–oxygen biofuel cells [138]. To provide enhanced electronic conduction the electrodes were based on enzyme encapsulation in sol–gel SiO_2 matrixes and incorporated carbon nanotubes (CNTs) in the matrix. The SiO_2 matrix was designed to be sufficiently porous that both glucose and O_2 have access to the enzymes and yet provides a protective cage for immobilizing the bio-molecules without affecting biological function. Voltammetry indicated that the effect of the SiO_2 matrix on mediator diffusion was minimal, although for one mediator, 2, 2'- azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, chemical modification of the solvent phase with polyethylene glycol was necessary. The polyethylene glycol addition resulted in a more uniform dispersion of the CNTs. The enzymes maintained their biocatalytic activity in the sol–gel matrix. A glucose–O_2 biofuel cell based on nanostructured SiO_2 sol–gel/CNT composite electrodes generated approximately $120\mu Wcm^{-2}$ at 0.24V at room temperature.

Another type of sol–gel/organic hybrid composite material based on the crosslinking of the natural polymer – chitosan – with (3-acryloxypropyl) dimethoxymethylsilane was developed for the fabrication of an amperometric H_2O_2 biosensor [139]. The composite film was used to immobilize horse radish peroxidase (HRP) on a gold disk electrode. With the aid of a catechol mediator, the biosensor had a fast response of less than 2 s with linear range of 5.0×10^{-9} to 1.0×10^{-7} mol L^{-1} and a detection limit of 2×10^{-9} mol L^{-1}. The biosensor retained approximately 75% of its original activity after about 60 days of storage in a phosphate buffer at 4°C.

The preliminary results of immobilization of the glucose oxidase (GOx) in silica gel on an oxygen electrode were also reported. The influence of the components of the casting solution (gel precursor, pH of the enzyme solution, sol–water ratio) on the electrode response was investigated [140].

The interest for organic–inorganic sol–gels develops into the incorporation of organic polymers in the inorganic sol–gel which can lead to new composite materials possessing the properties of each component that would be useful in particular applications. Incorporation of organic polymers, especially those with amino or amide groups, allows the formation of molecular hybrids often stabilized by strong hydrogen bonding.

The preparation of a platinum/multi-walled carbon nanotube (Pt/MWNTs) nanocomposite as glucose biosensor it was in this order mentioned [141]. Platinum nanoparticles were grown by electrodeposition onto MWNTs directly with the average diameter of the nanoparticles about 30–40 nm. The resulting Pt/MWNTs material presents new capabilities for electrochemical devices by using the synergistic action of Pt nanoparticles and CNTs. The immobilization of glucose oxidase onto electrode surfaces was carried out by chitosan-SiO_2 gel. Thus, the synergistic action of Pt and MWNTs and the biocompatibility of chitosan-SiO_2 sol–gel induce excellent electrocatalytic activity and high stability. The resulting biosensor exhibits good response performance to glucose with a wide linear range from 1 μM to 23mM and a low detection limit 1 μM. The biosensor also shows a short response time (within 5 s), and a high sensitivity (58.9 $\mu AmM^{-1}\ cm^{-2}$). For this hybrid nanocomposite it was provided a new electrochemical platform for designing a variety of bioelectrochemical devices with high sensitivity and good stability.

5.2. Biosensors for Total Cholesterol Estimation

As it is well known total cholesterol estimation is very important for patients suffering from heart diseases, hypertension, arteriosclerosis, cerebral thrombosis and other disorders that require continuous monitoring of total cholesterol. In this context, biosensors have recently gained much attention as they provide easy operation, accuracy, sensitivity and selectivity for cholesterol estimation. Several matrices such as nanomaterials, conducting polymers, sol–gel films and self-assembled monolayers have been used for fabricating a cholesterol biosensor *via* layer-by-layer technique, physical adsorption, hydrogel or sol–gel entrapment, electro-polymerization entrapment, cross-linking and covalent techniques, etc. [142-152].

The sol–gel-derived silica prepared using tetra-ethyl-orthosilicate (TEOS) precursor has been found to be more reactive towards the condensation reaction and has high affinity towards enzymes as compared to other precursors such as tetra-methyl-orthosilicate (TMOS), etc. [128].

Cholesterol esterase (ChEt) and cholesterol oxidase (ChOx) have been immobilized *via* glutaraldehyde as a cross-linker onto sol–gel-derived silica (SiO_2)/chitosan (CHIT)/multi-walled carbon nanotubes (MWCNT) - based nanobiocomposite film deposited onto indium-tin-oxide (ITO) glass for estimation of esterified cholesterol [153]. The realized ChEt–ChOx/MWCNT/SiO_2–CHIT/ITO bioelectrode, characterized using Fourier transform infrared

(FTIR), scanning electron microscopy (SEM) and electrochemical techniques, shows response time of 10 s, linearity as 10–500 mg/dL for esterified cholesterol (cholesterol oleate), sensitivity as 3.8μA/mM and shelf-life of about 10 weeks under refrigerated conditions. The value of Michaelis–Menten constant (*K*m) estimated as 0.052mM using Lineweaver–Burke plot indicates high affinity of ChEt and ChOx to cholesterol oleate.

Sol–gel-derived silica – chitosan (SiO_2 – CHIT) hybrid biocomposite exhibits interesting biosensor characteristics but very small response current were observed [154, 155]. The incorporation of multiwalled carbon nanotubes (MWCNT) into CHIT–SiO_2 hybrid film is likely to result in improved sensing characteristics. MWCNT enhance electro-catalytic activity due to presence of edge-plane-like sites located at both ends and in the defect region. Also, chitosan as a natural cationic biopolymer attracted much interest owing to its interesting properties such as biocompatibility, non-toxicity, low-cost, good film forming ability, high mechanical strength and high hydrophilicity. Moreover, the presence of amino and hydroxyl groups in CHIT facilitates immobilization of enzymes *via* covalent binding for biosensor application.

There are reports on integration of MWCNT with a biopolymer such as CHIT to fabricate biosensors to gain synergistic action using organic–inorganic hybrid nanobiocomposites. Tan *et al*. have fabricated a free cholesterol biosensor based on TEOS sol–gel CHIT–silica and MWCNT [156].

Other results report on studies relating to the immobilization of ChEt and ChOx immobilized onto SiO_2–CHIT/MWCNT bionanocomposite film deposited onto ITO coated glass for detection of total cholesterol using differential pulse voltammetry (DPV). DPV technique is more advantageous because it yields both peak shaped response and considerable reduction of undesirable currents contribution [157].

The use of such hybrid matrices provides a good microenvironment for the enzyme immobilization and help to avoid cracking of the gel macrostructure because the stabilizing organic groups in the developing matrix enable structural relaxation as well as a large extension of the stable pH region [158]. The study presents the formation of micropatterned sol-gel structures containing active proteins by patterning with polydimethylsiloxane (PDMS) microchannels. To transport sol solution efficiently into the hydrophobic PDMS microchannels, a hydrophilic-hydrophobic block copolymer was used to impart hydrophilicity to the PDMS microchannels. Poor adhesion of the micropatterned gel structure onto glass slides was improved by treating the glass surface with a polymeric substrate. To minimize cracks in the gel microstructure, hybrid matrices of interpenetrating organic and inorganic networks were prepared containing the reactive organic moieties polyvinylalcohol or polyvinylpyrrolidone. Retention of biochemical activity within the micropatterned gel was demonstrated by performing immunobinding assays with immobilized immunoglobulin G (IgG) antibody. The potential application of microfluidics technology to immobilized-enzyme biocatalysis was demonstrated using PDMS-patterned microchannels filled with trypsin-containing sol-gels. The work also provides a foundation for the microfabrication of functional protein chips using sol-gel processes.

5.3. Sol – Gel Method in Manufacture Surface Plasmon Resonance Biosensor

Various surface plasmon resonance (SPR) biosensors have been developed and applied for the detection and identification of specific analyte in the areas of biotechnology, environmental protection, medical diagnostic, drug screening, food safety and security [159].

A SPR biosensor system with magnetic beads was developed to detect the combination of antigen with antibody [160]. Owing to the difficulties to achieve complete dissociation of magnetic beads from the Au film due to the direct contact between magnetic beads and the Au film the titania sol–gel matrix was constructed on Au thin films to prevent the direct contact of the magnetic beads and the Au film. This determines also the easy regeneration and the sensitivity enhancement of the SPR biosensor.

The silica sol–gel process is usually carried out in acidic condition, which is hostile to the activities of biomolecules. At the same time, the silica sol–gel-derived matrix is fragile and easy to shrink, crack and desquamate from the electrode surface. As a result new sol–gel materials were thought for biosensor construction.

Thin films of crystalline TiO_2 nanoparticles are potential candidates for sensing applications having extremely large surface-to-volume ratio and peculiar surface properties of nanocrystals, which are expected to be beneficial for gas sensing purposes for example. TiO_2 thin films have also demonstrated good sensing properties towards humidity, oxygen and organic vapor, as well as for interfering gases such as CO, NO_2 and benzene.

The titania sol–gel matrix has been reported to immobilize enzymes in conductive polymers for electrochemical biosensor constructions. Titania is a kind of nonsilica material easily obtained from the sol–gel process being an alternate support material to silica for column packing in high performance liquid chromatography (HPLC) because of its high chemical stability, enough rigidity, and amphoteric ion-exchange properties [161, 162].

Usually, TiO_2 sol–gel, which was hydrolyzed in acidic conditions, was also not suitable for immobilizing enzymes because of the denaturation of enzymes under such low pH surroundings. Dry fracture of the gel membranes was also an obvious drawback for retaining the biosensor stability. Additionally, some membranes calcined at high temperature in the process of sol–gel fabrication before biomolecules immobilization prevented the biomolecules to be entrapped in the gel directly.

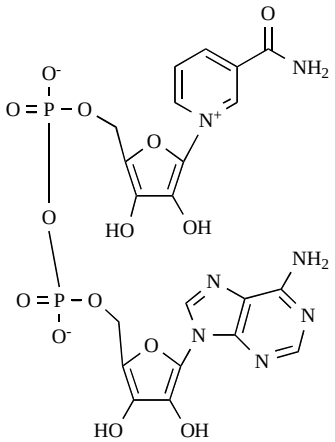
The achievement of an immunoassay of heat shock protein 70 (Hsp 70) by surface plasmon resonance (SPR) biosensor with magnetic beads immobilized on the Au film, it was reported [163]. The sol–gel-derived titania-modified Au film retained the bioactivity and provided for long-term stability of the biomaterials in storage. The presented vapor deposition method simplified the traditional sol–gel process and prevented the cracking of conventional sol–gel-derived

glasses. In the presence of titania sol–gel matrix as the safeguard, the SPR biosensor with magnetic beads exhibited a satisfactory response concentration range of Hsp 70 from 0.20 to 30.00μgml^{-1}. Compared to the measurement without modification of titania sol–gel matrix, larger resonant wavelength shift and sensitivity enhancement of the biosensor were obtained simultaneously.

Another titania sol–gel inorganic–organic hybrid nanocomposite film was prepared to fabricate a sensitive tyrosinase biosensor for the amperometric detection of trace phenolic compounds without additional electron mediators [164]. The used acetylacetone worked as a complexing ligand to chelate with Ti atom and to prevent sol from agglomerating during the hydrolysis procedure, then the $Ti(OBu)_3$ (acac) was used as precursor to synthesize TiO_2 sol–gel. Hence, the pH of the titania solution could be adjusted over a wide range by varying the buffer solution in the hydrolysis process to the value which was optimum for retaining tyrosinase activity and such a membrane was stably attached onto the surface of a glassy carbon electrode (GCE). Amperometry coupling tyrosinase with mediators is one of the most sensitive measurements with rapid response. The results showed that TiO_2 sol–gel matrix could also act as an effective promoter for the electron transfer between quinones and the electrode as well as co-immobilized mediator. This performed titania matrix supplies a good environment for enzyme loading which resulted in a high sensitivity of 15.78μAμM^{-1}cm^{-2} for monitoring phenols with a detection limit of 1×10^{-8}M at a signal-to-noise ratio of 3. The TiO_2 sol–gel derived biosensor exhibited a fast response less than 10 s and a good stability for more than 2 months.

5.4. Sol – Gel Technique Performing Fluorescence-Based Glucose Sensors

Problems with existing devices based on electrochemistry have encouraged alternative approaches to glucose sensing in recent years, and those based on fluorescence intensity and lifetime have special advantages, including sensitivity and the potential for non-invasive measurement when near infrared light is used. Several receptors have been employed to detect glucose in fluorescence sensors, and these include the lectin concanavalin A (Con A), enzymes such as glucose oxidase, glucose dehydrogenase and hexokinase/glucokinase, bacterial glucose-binding protein, and boronic acid derivatives (which bind the diols of sugars). The techniques include measuring changes in fluorescence resonance energy transfer (FRET) between a fluorescent donor and an acceptor either within a protein which undergoes glucose-induced changes in conformation or because of competitive displacement; measurement of glucose-induced changes in intrinsic fluorescence of enzymes (e.g. due to tryptophan residues in hexokinase) or extrinsic fluorophores (e.g. using environmentally sensitive fluorophores to signal protein conformation). Noninvasive glucose monitoring can be accomplished by measurement of cell autofluorescence due to *nicotinamide adenine dinucleotide* (the NAD(P)H coenzyme with two nucleotides joined through their phosphate groups, with one nucleotide containing an adenine base and the other containing nicotinamide – used as substrate of enzymes that add or remove chemical groups from proteins) (Fig. **9**), and fluorescent markers of mitochondrial metabolism can signal changes in extracellular glucose concentration.



Diphosphopyridine nucleotide (DPN^+), Coenzyme I

Fig. (9). Nicotinamide adenine dinucleotide Coenzime.

The problem of configuration of hexokinase-based glucose sensors for *in vivo* use by studying its encapsulation in porous sol–gels based on tetramethyl orthosilicate (TMOS), with or without covering membranes, it was studied [165].

Sol–gels have several potential advantages for glucose sensors intended for implantation in the body, including the provision of a matrix for immobilizing and containment of the enzyme, protection and excluding interfering substances such as fluorescence quenchers from the biological medium, favorably altering the enzyme kinetics (e.g. extending the linear range) and providing a biocompatible medium. Sol–gel immobilized yeast hexokinase retains its activity, showing an approximately 20% decrease in intrinsic fluorescence in response to saturating glucose concentrations. The maximal response of hexokinase in a monolithic sol–gel matrix was increased to about 40mM glucose (cf. 0.8mM for enzyme in solution) and the *K*m to about 12.5mM (cf. 0.3mM for free hexokinase). Application of an outer membrane consisting of 5% poly(2-hydroxyethyl methacrylate) extends the linear range to up to 100mM and the *K*m to about 57mM glucose. Unlike hexokinase in solution, sol–gel encapsulated hexokinase responds to glucose in serum with a decrease in intrinsic fluorescence comparable to that observed in serum free buffer, whether or not coated with an outer membrane. Techniques for encapsulation of *in vivo* fluorescence sensors are also in their infancy, at least as far as testing in humans or animals are concerned. Also, sol–gel immobilization and implanted fibre-optic probes are amongst the approaches that seem to have promise. More improved fluorophores for use in biological systems are in study, and in this respect quantum dots are already making an impact. Several fluorescent nanocrystal variants of quantum dots will emerge in the next few years and are likely to be incorporated in sensors. There is no doubt that fluorescence technologies have considerable promise for glucose sensing.

5.5. Sol – Gel Technique and Carbon Nanotube in Performing Biosensor

Extensive research studies on carbon nanotubes (CNTs) revealed their unique properties [166]. Their electrical and

other properties have been exploited for a variety of applications that include electrochemical sensors [167, 168]. CNTs have been employed as an electrode modifying material on the surface of glassy carbon, graphite, carbon fiber, gold, and platinum electrode towards fabrication of electrochemical sensors. Modified electrodes (MEs) with CNTs and supporting molecules as a binder or ion-exchanger have been used for the detection of many biomolecules, such as glucose, dihydronicotinamide adenine dinucleotide, hydrogen peroxide and amino acids [169-172]. However, the surface hydrophobicity and poor solubility of CNTs in common organic solvents limit their utility for applications. Functionalization of CNTs on their side walls as well in open ends makes them useful for the fabrication of MEs. Various MEs have been fabricated with functionalized CNTs and used for the electrochemical determination of bioanalytes. CNTs were functionalized with a conducting polymer and used as electrochemical sensors [173]. Polyaniline (PANI) was grafted onto multiwalled carbon nanotubes (MWNT) and used for the adsorptive reduction and determination of Celecoxib [174]. MWNTs were non-covalently wrapped by a cationic polymer and loaded into electrospun polymer nanofibers and a glucose sensor was fabricated [175]. Functionalized CNTs were also incorporated with metal nanoparticles (MNPs) towards fabrication of sensors. MNPs have attracted significant attention because of their unusual size-dependent optical and electronic properties. Among MNPs, gold nanoparticles (AuNPs) possess good catalytic activity for a number of reactions. The hydrophobic surface of CNTs can be modified or functionalized with adequate groups and subsequently MNPs could be loaded onto the surface of CNTs. AuNPs have been widely used for the construction of biosensors due to their excellent ability to immobilize biomolecules and at the same time retain the biocatalytic activities of the tested biomolecules. AuNPs could be anchored onto the surface of functionalized CNTs. AuNPs were self-assembled on a thiol-terminated sol–gel derived silicate network and a sensor was developed for the selective and sensitive detection of NADH etc. [176]. As was mentioned before natural polymers such as chitosan can be mixed with sol–gel material and such composites are less brittle and more biocompatible [177, 178]. These composites facilitate the electron transfer process between the biomolecules and the electrodes.

In general, sol–gel derived materials are electrical insulators with low charge transfer efficiency, poor mechanical stability and low resistance that limit their electrochemical applications [179, 180].

While silica imparts useful properties for the sensors, an additional material is required to have adequate electron transduction to the electrode. In this order MWNTs have been incorporated into sol–gel matrix to improve the electrical conductivity [181].

Considering the advantages of sol–gel based organic–inorganic hybrids, electrical properties of CNTs and catalytic properties of AuNPs, composites comprising of CNTs, silica and AuNPs were considered to have improved electrochemical sensor characteristics.

The preparation of CNT–silica–AuNP composite and its utility for electrochemical sensing of ascorbic acid (AA), it was reported [182-184]. AA is one of the most important water soluble components present in fruits and vegetables. AA is used in large scales as an antioxidant in food, animal feed, beverages, pharmaceutical formulations and cosmetic applications. Because of the biological and technological importance of AA, studies have been pursued to establish rapid and sensitive methods for the reliable determination of AA. The determination of the AA content was performed with several methods, such as titrimetry, fluorimetry, flow injection analysis, spectrophotometry and liquid chromatography.

Electrocatalytic oxidation at modified electrodes has been proved to be an important approach for the determination of AA. Electrochemical methods are simple and inexpensive. In the electrochemical process (Fig. **10**), AA can be easily oxidized to dehydroascorbic acid through a two-electron and one-proton process.

Subsequently, the irreversible hydrolysis could result in an electro inactive product, 2, 3-diketogluconic acid. The usefulness of various modified electrodes has been demonstrated for the electrochemical determination of AA.

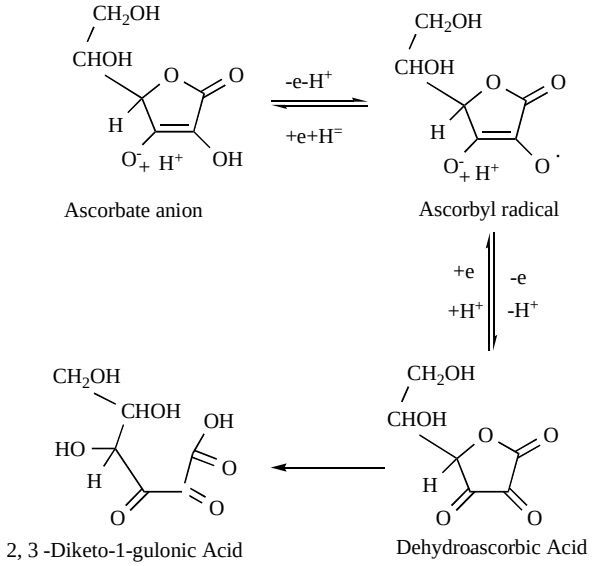


Fig. (10). Oxidative metabolism of ascorbic acid.

The literature mentions a screen-printed electrode modification with o-amino phenol film for the fabrication of an amperometric sensor for AA [185]. It was expected that the combined presence of CNTs, sol–gel silica and AuNPs to provide synergistic influences on the sensor performances. The preparation of a nanocomposite, comprising of MWNTs, AuNPs and silica and its use as an electrode modifier for the fabrication of AA sensor have been reported [184]. The modified electrode, GC/MWNT–silica–AuNPs, was fabricated by modifying the surface of multiwalled carbon nanotubes with silica networks and subsequent electrodeposition of gold nanoparticles (AuNPs). The morphology of the GC/MWNT–silica-NW–AuNPs modified electrode (ME) was analyzed by field emission scanning electron microscopy (FE-SEM). Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were used to evaluate the electrochemical performances of GC/MWNT–silica-NW–AuNPs-ME. Ascorbic acid (AA) was found to be

electrocatalytically oxidized at GC/MWNT–silica-NW–AuNPs-ME due to the synergistic influence of MWNTs, silica-NW and AuNPs. The MWNT–silica-NW–AuNPs-ME sensor electrode presents a high sensitivity (8.59μA/mM) and selectivity for AA in the presence of dopamine (DA). Differential pulse voltammetry revealed that the sensor electrode exhibited excellent selectivity for AA in the presence of large excess of DA with a large potential separation of ~0.26 V. The effective electrocatalytic activity, excellent peak resolution and ability for independent determination of DA in the presence of AA reveal that MWNT–silica-NW–AuNPs-ME is suitable for simultaneous and selective determination of DA and AA.

It is obvious that the enzyme-modified electrode is the fundamental component of amperometric biosensors and biofuel cells [186]. The selection of appropriate combinations of materials, such as: enzyme, electron transport mediator, binding and encapsulation materials, conductive support matrix and solid support, for construction of enzyme-modified electrodes governs the efficiency of the electrodes in terms of electron transfer kinetics, mass transport, stability, and reproducibility. The electrodes based on enzyme encapsulation in sol–gel SiO_2 matrixes and incorporated CNTs in the matrix provide enhanced electronic conduction. The SiO_2 matrix was designed to be sufficiently porous that both glucose and O_2 have access to the enzymes and yet provides a protective cage for immobilizing the bio-molecules without affecting biological function.

Voltammetry indicated that the effect of the SiO_2 matrix on mediator diffusion was minimal, although for one mediator, 2, 2 γ-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, chemical modification of the solvent phase with polyethylene glycol was necessary. The polyethylene glycol addition resulted in a more uniform dispersion of the CNTs. The enzymes maintained their biocatalytic activity in the sol–gel matrix. A glucose–O_2 biofuel cell based on nanostructured SiO_2 sol–gel/CNT composite electrodes generated approximately 120μWcm^{-2} at 0.24V at room temperature. Another new type of sol–gel/organic hybrid composite material based on the crosslinking of the natural polymer chitosan with (3-acryloxypropyl) dimethoxymethylsilane was developed for the fabrication of an amperometric H_2O_2 biosensor [138].

The composite film was used to immobilize horseradish polymerase on a gold disk electrode. With the aid of a catechol mediator, the biosensor presented a fast response of less than 2 s with linear range of 5.0×10^{-9} to 1.0×10^{-7} mol L^{-1} and a detection limit of 2×10^{-9}mol L^{-1}. The biosensor retained approximately 75% of its original activity after about 60 days of storage in a phosphate buffer at 4°C.

6. CONCLUSIONS

The sol-gel technique is one of the fastest growing fields of contemporary chemistry, the main advantage being the fact that it offers an alternative approach to conventional production of a wide range of materials which possess interesting features that may be exploited in various practical applications. The versatility of sol–gel chemistry enables us to manipulate the characteristics of material required for particular applications.

The revolution in the area of sol-gel-derived materials started since the demonstration that these materials may be used to encapsulate biological species such as enzymes, antibodies and other proteins in a functional state. Typical applications of sol-gel biomaterials include also selective coatings for optical and electrochemical sensors and biosensors, stationary phases for chromatography, immunoadsorbent and solid-phase extraction materials, controlled release agents, solid-phase biosynthesis, and unique matrices for biophysical studies.

This review focuses on the importance of sol–gel technology for preparing bioactive materials for biomedical applications. Sol–gel derived materials have proved to be very suitable as host of biologically active entities, since they are porous and the size and size distribution of pores can be controlled. Thus, they may serve as analytical devices for biocatalysis, biosensing and as drug release systems.

The sol - gel method in polymer science has shown the greatest potential in the design of novel polymeric biomaterials with advanced properties and functionalities. The growing number of such sol - gel materials more enables researchers to explore promising structures with better performance than their conventional polymeric counterparts. Thus, they can be used as biomaterials or they show responsiveness to external stimuli and can direct the design of biomedical devices for better spatial and timely control.

Like other scientific disciplines, the sol - gel biomaterials area provides both opportunities and challenges. From an applications point of view, two main questions arise: firstly, how can be applied the novel properties to design a medical device, and secondly, how can these novel properties intimately integrate with currently used medical devices? Although the analysis of chemical, physical and biological properties of sol-gel biomaterials seems to be challenging, their complex properties also provide opportunities in producing materials with excellent performance.

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