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SOL-GEL THIN FILM BASED SENSORS AND BIOSENSORS

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ABSTRACT

A wide variety of enhanced and modified sol-gel bulk and thin film processes for the development of sensors and biosensors have been witnessed in the last two decades. The porous silica glass material prepared by the sol-gel process is a promising host matrix for the encapsulation of various biomolecules viz. proteins, antibodies, enzymes. The entrapped biomolecules have shown to retain their functionality in the sol-gel matrix just like in solution by retaining their inherent properties. Therefore, this property of sol-gel has been widely utilized to form sensitive layers for sensing pH, gases, biomarkers, ionic species and solvents. Sol-gel derived thin films with entrapped biomolecules have been used as bio-recognition element for development of biosensors. The biosensors developed so far, has exhibited favorable sensitivity and response time towards the target analytes. The present review focuses on research carried out in the past years for the development of sensors and biosensors using sol-gel thin film entrapped biomolecules and their utilization as a sensitive membrane / bio-recognition elements for the construction of highly selective biosensors.

KEYWORDS: *Sol-gel, thin film, immobilization, biosensor*

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INTRODUCTION

A biosensor is composed of a bio-recognition element incorporated with an electronic transducer element. Enzymes and antibodies, play a vital role in clinical diagnosis because of their specificity and selectivity towards the analytes. Therefore, they are well recognized in the development of biosensors. Later, these bio-recognition elements are coupled with transducers (primarily optical, electrochemical, thermal or piezoelectric), which converts the recognition phenomenon into a significant signal [1]. Fluorescent probes or suitable enzymes are also utilized for the purpose of augmenting and generating quantifiable signals. After transduction, the signal is digitized for recording and storing purpose and could be viewed on a computer. Immobilization of biomolecules in suitable matrices or platform is an inevitable procedure to help these enzymes and antibodies gain better stability. Immobilization techniques include encapsulation, entrapment, adsorption, covalent linkage and cross-linking. The nature of the support or the matrix which holds the biomolecule, decides the method of immobilization. Among the several types of matrices like polysaccharides, polyamides, sol-gel and anhydride based copolymers, sol-gel processed glass materials have shown to serve better for immobilization of both antibodies and enzymes [2-3]. Early investigators observed that hydrolysis and polycondensation of precursors like tetraethyl orthosilicate (TEOS) / tetra methyl orthosilicate (TMOS) followed by aging and drying under ambient atmosphere, yield a glass like material [4]. The properties of the final glass depend upon the ratio of silanes, pH, catalyst and drying time [5]. The sol-gel processing is performed at room temperature and thus, protects the biomolecules against denaturation which is desirable for biosensor applications [6]. Prior to gelation, the sol is ideal for the preparation of thin films by using coating techniques viz. dip coating, spinning or RF Sputtering [7-8]. It is imperative to obtain a stabilized environment with desired properties in sol-gel thin film for sensing applications, as the changes in environment are likely to affect the overall sensing response. Entrapped molecules like enzymes, proteins and other sensing molecules need suitable environment during storage. A very important factor in the development of enzyme-based biosensors is the immobilization of enzymes on suitable transducers. Over the past few decades sol-gel technology has established itself in offering interesting techniques in the field of biosensor technology, biomaterials and nano particle formation [9]. Sol-gel derived thin film not only meets the challenge in the development of competitive biosensors but also provides user-friendly, accurate, reliable, portable and inexpensive technology[10]. The process utilizes less power and produce less potentially toxic waste. Moreover, Advances in manufacturing techniques with miniaturization enhances the portability of on-site operation of biosensors in larger scale [11]. In this paper we present a detailed review of the applications of sol-gel derived thin film based sensors and biosensors, reported in the past two decades. Special attention is devoted to antibody and enzyme based biosensors, as this field has been experiencing a rapid growth due to the increasing demand for stable, robust and specific devices for medical diagnostics, *in vivo* monitoring, food industries, environmental control and biotechnology [12].

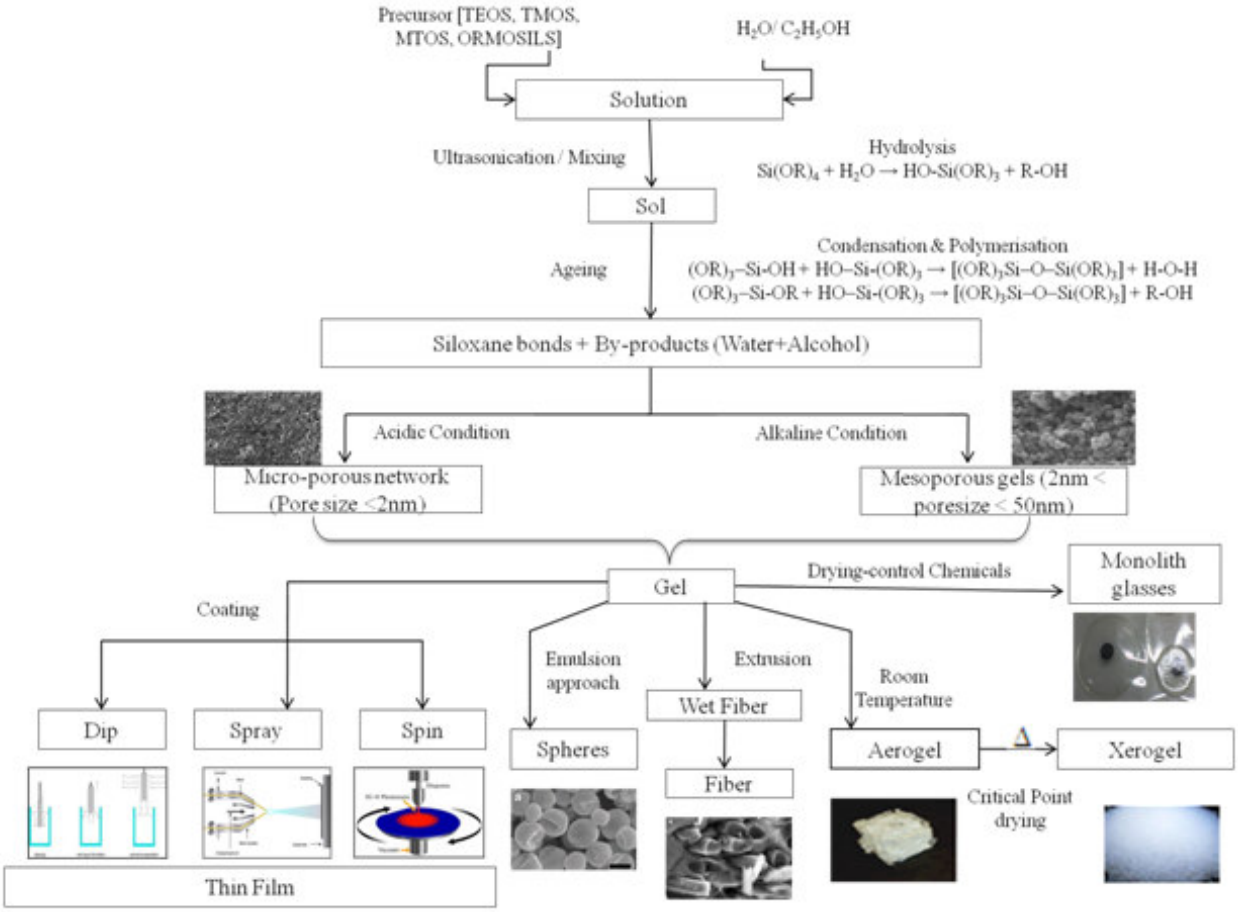
MATERIALS AND METHODS

Sol-gel processes

Immobilization of enzyme within silica matrices were first reported about fifty years ago by Dickey [13]. However the development of sol-gel as biomaterials, started in the early nineties [14]. The properties of entrapped enzymes in TEOS-derived sol-gel matrices were initially analysed by Larry et al. [15]. The procedure was rapidly extended to antibodies and other biomolecules, which facilitated the design of sol-gel based sensors, biosensors and bioreactors, as recently reviewed by several authors [16-17]. At present, various methods are being utilized to obtain a matrix for immobilization of the antibodies as sensing elements. Among all, sol-gel is undoubtedly the cheapest and comparatively the simplest process. The process comprises several steps which are well described in the literature [18-21]. Mennig et al., [22] have listed out the solubility of the precursor in the reaction media and the ability to promote the gel formation, as the requirements for sol-gel processing. The precursors that are used may be one of the following classifications: salts, hydroxides, oxides, complexes, acylates, alkoxides, and amines. Comparatively the most commonly used categories are the alkoxides as they react with water gently leading to better homogeneity, avoiding phase separation [23]. The concentration of silicon alkoxides and its type influence the rates of hydrolysis and condensation. This leads to the monomer being produced at different rates. The synthesis of SiO_2 based materials is mostly dependant on TEOS as precursor. As the TEOS concentration increases the solution's viscosity and the polymer's concentration also increases, without varying their shape or size significantly [24]. The mild conditions of the sol-gel process make it ideal for the immobilization of numerous organic, organometallic and biological molecules. Characteristics such as polarity, porosity and ion exchange capacity can be easily tailored by simple modification of the polymerization protocol. Sol-gel derived glasses are also porous in nature with high thermal and mechanical stability. They are reported to exhibit less photo degradation and are transparent to wavelengths above 250 nm, which makes them highly suitable for optical sensing applications [21]. Sol-gel glasses can be obtained in a variety of shapes and configurations (thin films, fibres, monoliths, powders, etc.) and be attached to most other materials [12]. Use of buffers (phosphate, succinate, acetate and oxalate) in sol-gel preparation has been reported to obtain stabilized and favorable sol-gel for protein encapsulation and biosensor applications. Entrapped proteins have been shown to retain their functionality in buffered sol-gel. The porous sol-gel matrix doped with analytical reagents has been successfully used to show the interaction with the target

analytes [25-35]. The pH of buffer plays important role in making the final solution to be close to neutrality in order to avoid denaturation of biological molecules. Also, depending on starting precursors and process conditions such as temperature, molar ratio (R) and pH, different products and morphologies can be obtained which is illustrated in Figure 1.

Figure 1
Schematic of sol-gel processing



The use of organically-modified silanes (ORMOSILS) for the development of modified sol-gel-derived materials has increased rapidly in recent years. These are organic-inorganic hybrid materials, which combine the properties of an inorganic glass and an organic polymer. High optical quality nano composite materials with desirable properties can be prepared using ORMOSILS [36]. TEOS-derived, alcohol free sol-gel has been synthesized, for the design of biosensors, by the entrapment of living cells, proteins and antibodies which demand biocompatibility. The results obtained from their work, showed that these bioactive materials had optimized optical properties [37].

SOL-GEL THIN FILM PROCESSING

Sol-gel process mainly involves two steps i.e. hydrolysis and condensation reaction of organometallic compounds in the suitable solvent. Thin films are derived during the sol phase and their characteristics can be easily modified and customized in solution stage by using buffers and other additives [21]. It has been reported that films can be modified by using very small amount of embedded functional molecules. They are advantageous in exhibiting transparency and uniformity. In addition, thin films are reported to possess appreciable mechanical resistance and are less prone to cracking if processed with suitable additives viz. triton X, glycerin. Thin films are formed by draining through gravitational or centrifugal techniques accompanied by vigorous drying. Thin films which have a thickness of usually < 1 µm have been obtained by: spin-coating, dip-coating or spray-coating techniques [38] and these are treated as the most technologically significant sol-gel configuration, which represents a vital area for the fabrication of optical sensors. Spin coating and dip coating techniques are utilized in developing single or double coated thin films. Roll coating is identified as another coating technique which is broadly applied for industrial coatings, targeting especially the flexible substrates. It can make coatings

with high speed (200 feet/minute) [39]. Each technique offers significant advantages and features required for immobilization of enzymes, proteins and other sensing molecules. Drying is the decisive component for the shape of the fluid profile and the continuance of the deposition process. The coating technique henceforth is decided by the specific applications, the biosensors are intended for [40].

1. THIN FILM CHARACTERISTICS

The important consideration for thin film formation is the uniformity and thickness of the film, its adhesion to the substrate, resistance to crack, stable internal environment and minimal leaching of entrapped molecules [25]. Juan Li et al., [41] carried out experiments with sol-gel films of varying thickness. The effect of changes in the film thickness was analyzed on the electrode performance. The thickness of sol-gel film was varied by changing the amount of alcohol in the sol-gel solution while the volumes of the other compounds were fixed. There was a significant effect of the thickness of sol-gel film on the smoothness. The surface of the film appeared flat, without the fine cracks being visible to the naked eye. The thickness of the film also affected the response time, by hindering the diffusion of the mediator or the product through the film, the response time increases as the thickness of the sol-gel film increases [42]. The structure of films deposited from polymeric or inorganic precursors depends on factors such as size and structure of the precursors, relative rates of condensation and evaporation, capillary pressure, and substrate withdrawal speed [38]. Also, film properties are crucially dependant on several parameters such as, pH, aging time, dipping time, withdrawal speed and R value (water: precursor ratio). It was proven that stable films can be produced by increasing the R value, as a result of complete hydrolysis. Aging of the sols was studied by McDonagh et al., [43] and reported that the film thickness increased as the aging time increased, for different Molar ratios of Ethanol: TEOS and H_2O: TEOS. The results paved way for more in-depth understanding of thin film process rendered to the fabrication of higher quality sol-gel thin films.

2. PHYSICO-CHEMICAL PROPERTIES OF THIN FILMS

The sol-gel thin film structure and physico-chemical properties are important to meet the criteria for making it reliable for sensor applications. Various spectroscopic techniques have been utilized by several researchers to characterize the thin films. The spectroscopic ellipsometry (SE) a non-invasive technique, has been widely used to analyze the properties of thin films optically. Apart from the monitoring of real-time characterization and non-disrupting progress of thin-film, SE is also applied to analyze the transparency, porosity percentage and the dielectric properties. SE analysis for the porous thin films was accurately performed using techniques like linear calculation model and Effective Medium Approximation (EMA) [44]. McDonagh et al., [43] carried out experiments on ellipsometer to evaluate film behavior based on the dependency of the rate of hydrolysis and condensation on pH of the sol and R (molar ratio) value. It was demonstrated that there is a distinct increase in the thickness of the film with subsequent increase in pre-polymerization time of the sol. The experiments were conducted with increase in R value (2-6). The higher R values decreased the duration to achieve the stability of the film. Thickness and processing time was also observed which showed that surface quality of the thin films relies on the processing conditions. In continuation to previous research, McDonagh et al., [45] reported on characterization of oxygen-sensitive tetraethoxysilane (TEOS) and methyltriethoxysilane (MTEOS)-based films. The porosity of the film and sensor response times were reported for a range of films fabricated under different conditions. The behavior of the porosity, V_p, as a function of *R*-value and sol aging time is shown in Figure 2. It was observed that porosity decreases with increasing *R*-value for a given aging time, while, for a given *R*-value, porosity increases with increasing aging time. The porosity behavior of TEOS films as the ethanol content is varied, is shown in Figure 3. This indicates a decrease in porosity with increasing ethanol content of the sol. The enhanced diffusion coefficient of MTEOS (methyltriethoxysilane) films compared to TEOS films was reported and was discussed in terms of the relative oxygen solubility of the films.

Figure 2
Porosity with respect to sol-gel aging time for R values 2 and 4.

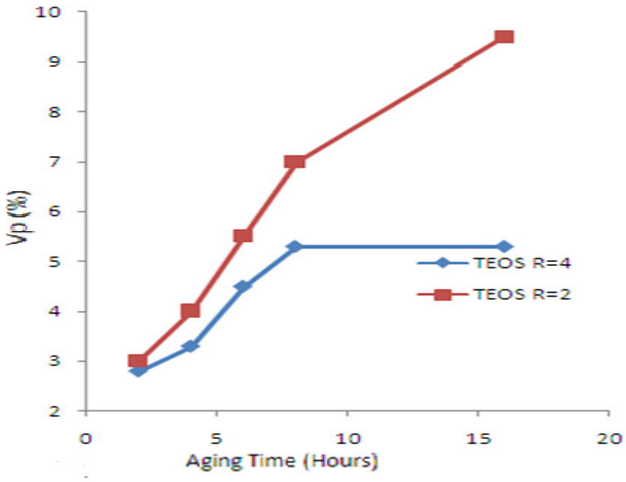
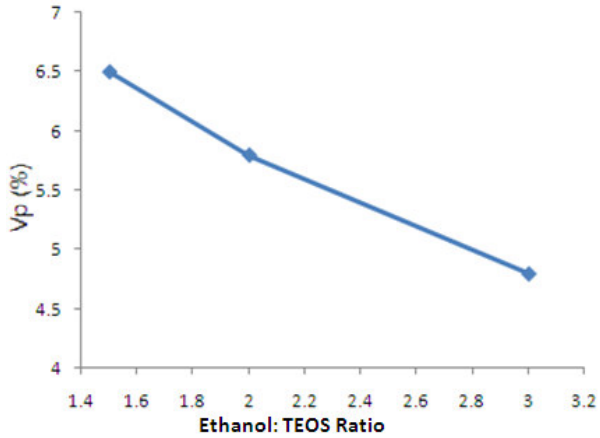
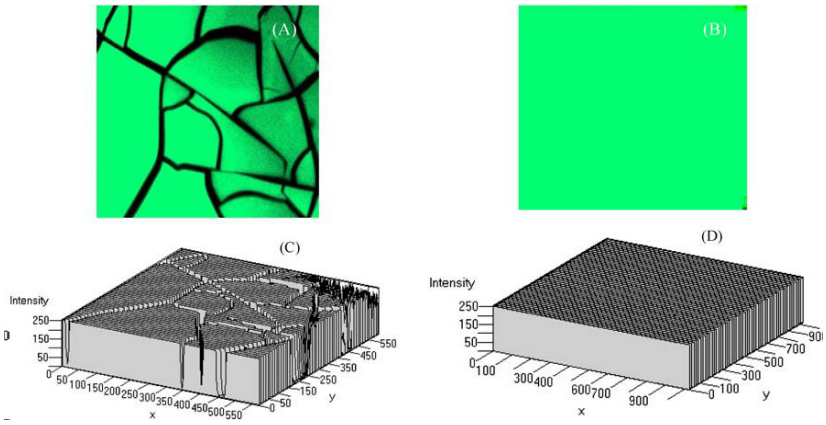


Figure 3
Thin Film Porosity with respect to Ethanol:TEOS ratio developed at pH=1, R=4 and aged for 6 hours.



Bhaskar et al., [24] utilized confocal microscope for visualization of the surface of thin films doped with fluorescent dyes: Pyranine and Hoechst33258. The thickness of the thin film was in the range of 1–10μm and confocal images showed in Figure 4 represent uniform distribution of fluorescent molecules. Confocal microscopy based visualization reported to be a useful tool for fluorescence based studies of thin films. Sol-gel coatings can also be easily combined with optical fibers or planar waveguides, providing intrinsic evanescent wave sensors; furthermore, thin films have great potential for miniaturization and also permit the possibility of preparing multi-layer configurations[26].

Figure 4
Confocal microscopic fluorescence images of sol-gel thin film



Confocal microscopic fluorescence images of sol-gel thin film containing H258 (A) Cracked (B) Smooth thin film. The depth profile of the films shown below (C and D) clearly shows the nature of sol-gel thin film and distribution of fluorescent probe [26]. For characterization of surface morphology, chemistry, thickness, and nanocrystallite size of thin films several advanced analytical techniques are also being utilized such as scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS), X-ray diffraction (XRD), atomic force microscopy (AFM), and high-resolution transmission electron microscopy (HRTEM) [42].

IMMOBILIZATION OF BIOMOLECULES

Enzymes are produced naturally in response to the biochemical pathways in the body. *In vitro* immobilization technique is used for the recovery and the efficient reuse of the enzymes during biocatalytic transformation [46]. Immobilized enzymes are well defined as "enzymes physically confined or localized in a certain defined region or space with retention of their catalytic activities, which can be used repeatedly and continuously". In the last half century, several methods of immobilization have been carried out and reported to be suitable for biomolecules. These techniques are either binding or inclusion onto natural or synthetic carriers / networks [47], or cross-linking of molecules [48-52]. The extensively used protocols for immobilization are (1) adsorption of sensing agents onto a solid substrate; (2) covalent binding which involves the formation of permanent chemical bonds between sensing agents and a support; and (3) encapsulation or entrapment of sensing agents within a polymeric matrix. The most preferred is a simple, uncomplicated methodology of immobilizing the biorecognition element, such that it maintains its affinity and is stable over time [53]. Covalent attachment procedures are attractive, but can be tedious, and demands comparatively overpriced reagents. It also calls for the presence of specific functionalities on the biomolecule. Procedures like polymer entrapment and adsorption of proteins repeatedly undergo desorption, and denaturation. The sol-gel method proves to be a means to incorporate biomolecule within inorganic/organic materials. Sol-gel films are captivative host materials for the following reasons: they are developed under ambient conditions; they present adjustable porosity; holds good thermal stability; exhibits optical transparency and entail simple entrapment protocols [14, 54]. Various research has been focused on encapsulating biomolecules within the porous sol-gel matrices which can be used as biosensors. The porous nature of the sol-gel material makes it as suitable platform for entrapping biorecognition elements and also paves way for easy accessibility and selective interaction with specific analytes [55]. Several attempts have been made to encapsulate antibodies for biosensor applications by various groups since the start of sol-gel process. The various methodologies to entrap antibodies into sol-gel in the past decade have been tabulated in Table I. Sol-gel coatings are being used in the development of biosensors, with promising results in terms of sensitivity and detection limit. The prospects of using sol-gel encapsulated biomolecules for development of improved biosensors were greatly brought forward by the feasibility of preparing thin films [56, 57]. Also, the past few years have seen continued emphasis on enzyme and antibody-based biosensors. The emergence of new recognition approaches, most notably those employing aptamers and phages are also one of the significant advancements of this field. Presently, research on the development of biosensing systems based on the enzymes is being carried out in the field of (1) enzyme immobilization, (2) integration of enzymes with specific nano-structures to intensify the electron transfer capabilities, and (3) enzyme engineering to enhance subtlety and immobilization. For biosensing based on affinity, few innovative approaches are focused on (1) aptamer-based biosensors, where the new aptamer structures are designed and conjugated with various physiochemical transducers, and (2) phages which are specific biorecognition structures that increase the specificity and enhance the stability of the biosensor [58].

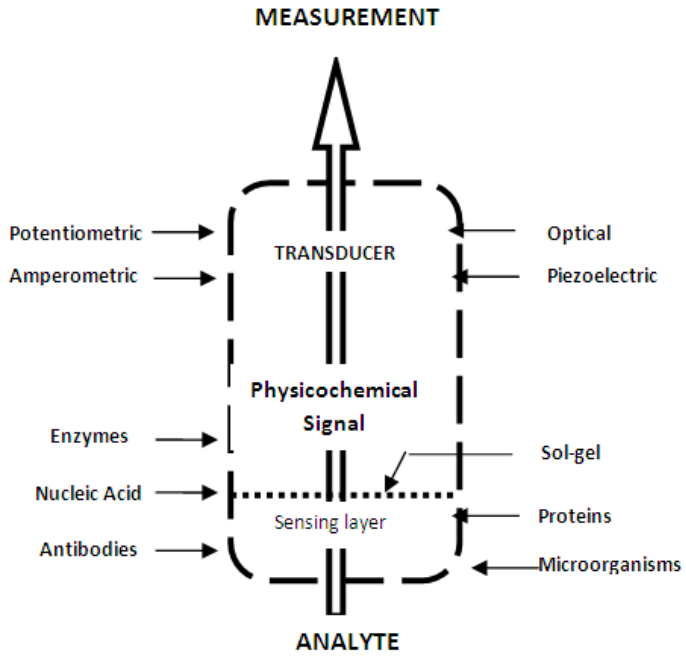
Table: I
Immobilization of antibodies via sol-gel process

Sol-gel Matrix	Immobilized antibody	Lifetime/Stability	Technique Used
Sol-gel doped with anti-isoproturon [72]	Isoproturon	two months	fluoroimmunoassay
Sol-gel matrices [94]	Atrazine Antibodies (Abs)	100% stability for at least 2 months	ELISA
Sol-gel [95]	Anti Trinitrotoluene	two months	Fluorescence
Sol-Gel [96]	Levonorgestrel Antibodies	few weeks at 4 °C 100% stability	Confocal fluorescence
Ormosil sol–gel membrane [97]	HRP-anti-hCG	90% of its initial- current after 30 days.	Amperometric responses
screen-printed carbon electrodes with biopolymer/sol-gel [98]	Horseradish peroxidase -labeled antibodies	94.7% of initial responses after 35 days.	Electrochemical analysis
Sol-gel [99]	Anti Cortisol	Few weeks	Fluorescence
TMOS based sol-gel [100]	Anti-levonorgestrel abs	Four weeks	Fluorescence

DEVELOPMENT OF SENSORS AND BIOSENSORS

It is now well established that a wide variety of enzymes and proteins retain their characteristic reactivity's and chemical functions when they are confined within the pores of sol-gel derived matrices. A substantial subpopulation of sol-gel entrapped bio-recognition elements remain accessible and can selectively interact/react with chemical or biological species (analytes) within the sample milieu [55]. Different transduction systems *viz* electrochemical, optical, piezoelectric have been employed for the development of sol-gel biosensors. A typical configuration of sol-gel based biosensor is shown in Figure 5.

Figure 5
sol-gel matrix containing suitable receptor biomolecule in sensing layer can provide suitable sensing layer for detection of several analytes.



The denaturation of proteins caused by the presence of low pH and an elevated amount of alcohol makes the conventional protocol of sol-gel thin film formation, unsuitable for encapsulation of biomolecules. Hence, addition of an appropriate buffer is used as a modified protocol, which maintains the pH [59-62]. Recent years witnessed huge progress in the biomolecules encapsulation in porous silica networks by altering the traditional methods and evolving mild polymerization conditions in order to maintain the indigenous structure and characteristic activities of the protein. The various processing methods and ready availability of a large number of precursor materials means that almost any glass composition can now be prepared by the sol-gel method. There are several literatures to support the immobilization of enzymes and other biological molecules within sol-gel films 63. Glezer and Lev et al. [64] designed a glucose biosensor by casting the GOx-doped V_2O_5 sol-gel-derived film onto the platinum-electrode transducer. Sol-gel vanadium pentaoxide (V_2O_5) possess high

conductivity and yielded a good response. Narang and coworkers [61] described the multiple-layer construction of glucose oxidase immobilized in between two sol-gel derived films. The sandwich construction was found to be favorable in defending the enzyme from the hostile environment encountered during sol-gel film processing. Pankratov et al. [65] constructed a multilayer "sandwich" made by placing a layer of enzyme between a carbon-ceramic composite matrix and a sol-gel derived silica film. The set-up provided versatile, easy-to-prepare, renewable amperometric biosensor with high sensitivity, selectivity and stability. A novel technique involving the antibody antifluorescein layer sandwiched by sol-gel thin film composite was developed by Jeffrey et al. [43]. The fluorescent hapten Me_2F was used to prove the viability of the antibody. The recognition property of the antibodies was stable for several weeks. An ideal storage in pH8 phosphate buffer at 4°C was implemented. Interestingly, the response time improved as a function of storage time. Roux et al.[66] synthesized porous silica gels and used for the encapsulation of antigens sourced from hydatid cyst fluid. The results demonstrated that the pores of the matrix are large enough to allow the diffusion of antibodies through the gel and enabled the antigen-antibody specific fixation, which was detected via the enzyme linked immunosorbent assays (ELISA). Sol-gel film provided equivalent specific fixation as observed in antigen solutions. Li et al. [67] designed a sensitive and selective biosensor by immobilizing HRP (horseradish peroxidase) on a carbon plate electrode. The thin film of sol-gel improved the stability and exhibited a good electrochemical response, by paving way for the mediator hexacyanoferrate (II) to shuttle electrons between the HRP in the silica sol-gel matrix and the CPE. This procedure demonstrated that the sol-gel technique is an attractive approach for the immobilization of enzymes which can be employed as electrochemical sensors and biosensors. A sol-gel silicate-based biosensor was developed by Toshio & Takashima [68] for sensing glucose by utilizing a composite membrane of sol-gel enzyme film and electrochemically generated poly(1,2-diaminobenzene) film with improved selectivity. The stability of the sensor was improved by exposing the enzyme layer to glutaraldehyde vapor. The glucose sensor responded rapidly (ca.15s) to glucose at 0.6 V (versus Ag/AgC1). The electro active species (L-ascorbate and urate) did not rend any interference to the responsiveness of the sensor. The same sol-gel procedure was employed as the preparation methodology for sensing galactose and cholesterol. Though the sensors responded rapidly the sensitivity of these sensors was comparatively inferior to that of glucose sensor. Similarly, Yang et al. [69] exploited a micro fabricated glucose biosensor by entrapping GOD in the sol-gel layer of thickness 5 A° and pore size of 2 mm thickness to detect hydrogen peroxide formed in the enzymatic reaction. There was a significant improvement in the reproducibility of the sensor for mass production. Wankhade et al. [101] synthesized Peroxide Sensor based on PVA-PANI nanocomposite, based on sol-gel and proved the efficiency of sensing peroxide. Otto et al. [70] developed three types of glucose biosensors, incorporating GOx i) in sol-gel sandwich configuration, ii) in sol-gel two layered configuration and iii) in a single layered configuration. The single layer configuration provided the best operational lifetime. Sorbitol was added to obtain more porous sol-gel and thus improve diffusion. The storage stability of all configurations exceeded 4 months if stored at 4°C. List of Biosensors developed using immobilized enzymes in sol-gel by various research groups has been tabulated based on their stability, shelf life and type of sensing an element used in Table II.

Table: II
Biosensors based on immobilization of enzymes in sol-gel thin film

Sol-gel composite matrix	Biological element	Analyte	Lifetime/ stability	Principle of Detection
Sol-gel thin film [29]	Tyrosinase	Phenol	73% of its initial activity for 3 weeks	Amperometric
AgNPs doped CNT film with hybrid sol–gel [30]	Myoglobin	Hydrogen peroxide	Favorable stability	amperometry
Sol-gel thin film layers [42]	Antifluorescein	Me2F	13 weeks	Fluorescence
Sol-gel thin film [62]	Cytcd1NiR	$NO2^-$	Several months	Absorption
Silica-carbon matrices [64]	Glucose oxidase	Glucose	Very low stability	Amperometric
Sol-gel enzyme generated poly (1, 2-diaminobenzene)[68]	GOD enzyme	Glucose	41% of its original activity after 30 days	Amperometric
Ru(dpp) doped sol-gel with sol–gel entrapped GOx [70]	Glucose oxidase	Glucose	Exceeds 4 months if stored at 4°C	Luminescence
sol-gel on the CNT-Pt base electrode [76]	cholesterol oxidase	cholesterol	stable	Electrochemical
Nylon membranes with TEOS based hydrosol-gel [80]	urease and phenol red	Urea	20 days	Change in color
Sol-gel-derived silicate / Nafion [81]	Tyrosinase	Phenolic compounds	74% of its initial activity after 2 weeks	cyclic voltammetric
Sol–gel and natural polymer chitosan film [82]	HRP	Hydrogen peroxide	85% of its original response after 1 month	Amperometric
Sol-gel chitosan/silica and MWCNTs [83]	Cholesterol oxidase	Free cholesterol	89% of its original response after 50-days	Amperometric
TMOS + (PhTMOS) + MTMOS and dopamine. [84]	(Fe(bpy)3)2+	Iron(II)	2 weeks	Chronoampero-metric
Sol–gel derived silicate material [85]	Calmodulin antagonists			Dissociation
CPE and a silica sol–gel film [86]	Hemoglobin	H_2O_2	93% of its original response after 3 weeks.	Electrochemical
sol–gel network [87]	Acetyl cholinesterase	Acetyl-choline	20 days	Fluorescence
Sol-gel-$Fe(C_5H_5)_2$:GOx:sol-gel [88]	Glucose oxidase	Glucose	30 days	Amperometric
Sol–gel entrapping matrix [89]	Glucose oxidase	Glucose	5 days	Amperometric
Sol-gel thin film [90]	Urease	Urea	>6 weeks (stored 4 ◦C)	Absorption
Sol–gelentrapped bromcresol purple [91]	Cholinesterase	Carbaryldichlorvos	3 weeks	Absorption
Sol–gel-immobilized on IDA electrode [92]	Urease	Urea	63% of its original activity after 25 days	Conductometric
Sol-gel-derived TiO_2 [93]	Glucose oxidase	Glucose	At least 1 month	Amperometric
Sol-gel derived PANI naocomposites [101]	Peroxidase	Peroxide	At least 1 month	Electro- Chemical

Physical adsorption, microencapsulation and physically entrapped sandwich (sol-gel: enzyme: sol-gel) techniques were described by Kumar et al. [71] in which the technique is used to co-immobilize the enzymes cholesterol oxidase (ChOx) and horseradish peroxidase (HRP) in a sol-gel film. These systems contributed an increased response time for the microencapsulated films in determining cholesterol. Chemiluminescent H_2O_2 sensor with rapid response, good reproducibility and long lifetime was designed by Wang et al. [60] by physically immobilizing HRP in sol-gel films. Zhang et al., [29] demonstrated the determination of phenols by immobilizing an amperometric tyrosinase enzyme into sol-gel solution. The tyrosinase maintained 73% of its initial activity, in the sol-gel thin film for three weeks when storing at 4°C in dry state. Similarly, Pulido et al. [72] reported the suitability of sol-gel powder entrapped with anti-isoproturon to determine isoproturon, which can determine the presence of pesticides in real samples. GOx-doped TMOS-derived, Ferrocenium (electron acceptor) integrated, sol-gel film was coated on a glassy carbon electrode by Audebert and co-workers to show incontestably the generation of biocatalytic currents in the presence of glucose. The gel retained of about 80% of the enzyme activity [73]. A biosensor for p-iodophenol, p-coumaric acid, 2-naphthol and hydrogen peroxide, based on the chemiluminescence (CL) reaction of the luminol-H_2O_2-horseradish peroxidase (HRP) system was developed by Ramos et al., [74] by microencapsulating HRP in a sol-gel matrix. Later, Kim et al. [75] developed an amperometric biosensor to determine phenolic compounds, by immobilizing tyrosinase in a thin composite film composed of silicate/nafion polymer. The biosensor showed excellent result by increasing the long term stability of the biosensor. Shi Q et al. [76] prepared carbon nanotubes decorated nanoplatinum which acts as an electrochemical biosensor for cholesterol. The immobilization of the cholesterol oxidase with sol-gel on the CNT-Pt base showed an increased rapidity in sensing cholesterol. Jeronimo et al. [77] employed carbonic anhydrase (CA) to develop an optical biosensor and determined the antiglaucoma agent acetazolamide. The enzyme and a pH sensitive dye, cresol red, were entrapped in overlapped sol-gel films, in a dual-layer format. CA catalyzed the dehydration of bicarbonate, which was inhibited by acetazolamide and resulted in color transition of cresol red. The developed optical biosensor displayed good sensitivity, reduced leaching and rapid response (60s).The immobilized enzyme showed stability when stored at room temperature after a

period of three months and was utilized for monitoring the enzymatic reaction, as well as its inhibition by acetazolamide. Recently a fluorescent biosensor for determination of herbicides, by entrapping fluorescent biomaterials in translucent matrixes obtained from a sol-gel process was reported Nguyen et al.,[78]. Ramanathan et al. [79] demonstrated that the OPH encapsulated on a silica matrix, acts as an efficient biosensor for paraoxon, an organophosphate, retained its catalytic activity for 60 days. Chuan et al.[30] used nanocomposite to modify the CNT layer electrodeposited by AgNPs, to construct active layers which was then coated with Mb-hybrid silica sol-gel (HSG). The technique proved itself as a specific biosensor for hydrogen peroxide. Two years later a biosensor by co-immobilizing urease and phenol red on nylon membranes with TEOS based hydrosol-gel was fabricated by Verma et al [80]. The Response time was correlated with the concentration of urea. The detection limit was achieved as low as 10^{-9}M with a response time of 2 min 29 seconds. The developed biosensor was stable for 20 days when kept at 4^0C. Sol-gel method has found unique applications in the development of sensors and biosensors, apparently distinguishing itself in its capabilities. Sol-gel thin films have proven a versatile and useful for entrapment of micro-organisms, antibodies, enzymes, nucleic acids and intrinsic receptors. It provides many advantages over other materials and methods utilized for entrapment of biomolecules for the development of sensors and biosensors. The applications of sol-gel thin film have been growing, although some of these applications discussed in the review did not directly lead to the development of biosensors. Furthermore, since sol-gel glasses are stable and nontoxic, are suitable candidates for development of devices suitable for in vivo and medical applications. A simple, rapid test based on sol-gel thin film biosensors would facilitate early diagnosis and easy management of diseases in clinical practices. The current tests usually used to detect several biomarkers are based on ELISA (Enzyme-Linked Immunosorbent Assay) which is time consuming complex procedure and requires skill to perform. The direct measurement of biomarkers using sol-gel entrapped sensitive layer on the suitable transducer would be one of the breakthrough in the field of medical diagnostics. Similarly, micro sensors have found recent applications in defense, aerospace and medical applications where sol-gel technology could play a potential role in the development of robust and accurate sensor systems. Sol-gel derived glass for the entrapment of sensing agents have potential advantages over other methods however, the diffusional limitations inside the porous network (in case of monoliths), reproducibility of results and sensitivity (in thin films) remains a challenge for the researcher. In recent years, a number of new sol-gel derived materials have been designed with the purpose of making the matrix more compatible with entrapped biological molecules.

CONCLUSIONS AND TRENDS

The present review focuses on the applications of sol-gel thin film for the development of sensors and biosensors. Sol-gel thin films doped with appropriate biomolecules could be utilized for sensing of variety of analytes. Moreover, uniformity, stability, long shelf-life of thin films remains a big challenge to make it a useful platform for the development of sensors and biosensors. New biocompatible silane precursors and processing methods based on glycerated silanes, sodium silicate, or aqueous processing conditions were primarily directed towards removal of alcohol by products by evaporation before the addition of proteins. Other approaches include the use of protein stabilizing additives such as organosilanes, polymers, sugars and amino acids (osmolytes) to silica to improve the protein stability. The applications of biocompatible sol-gel-derived matrices can be further extended and utilized in development of sol-gel sensors that are immune to reagent leaching and can be used for a long time period without changes in sensitivity and response time for the detection of multiple analytes. In addition to the technical improvements an important question that must be considered for the successful development of sol-gel thin-film based sensor and biosensor is the nature of competition offered by alternative techniques. The sol-gel biosensor will have to compete with conventional techniques viz. ELISA in medical diagnostics. Various techno-commercial aspects cost, ease of use, sensitivity, operational stability, robustness and high shelf life will decide the acceptance of sol-gel based biosensor in biomedical applications. The potential applications have obviously not yet been fully realized due to the lack of commercialization. Paying more efforts to understand the inherently complicated nature of sol-gel process and its mechanism, the gel microstructure and its nature on sol-gel thin film biosensors would facilitate early diagnosis and easy management of diseases.

REFERENCES

1. Nakamura., Isao. Current research activity in biosensors. Anal Bioanal Chem, 377 : 446–468, (2003).
2. Malitesta C., Palmisano F., Torsi L. Zambonin P.G. Glucose fast response amperometric sensor based on glucose oxidase immobilized in an electro polymerized poly (o-phenylenediamine) film. Anal. Chem, 62: 2735–2740, (1990).
3. Lee J.S., Nakahama S., Hirano A. A. New glucose sensor using microporous enzyme membrane. Sens. Actuators B (3): 215–219, (1991).
4. Hardy A.B., Gowda G., Mcmahon T.J., Riman R.E., Rhine Ebowen W. Ultrastructure Processing of Advanced Ceramics. In: J.D. Mackenzie D.R. Ulrich (Eds.), Wiley, Chapter 30: 407–428,1984.
5. Hench L.L., and West J.K. The sol-gel process. Chem. Rev., 90 (1): 33-72, (1990).

6. Collings A.F., Caruso F. Biosensors: recent advances. Rep. Prog. Phys. 60:1397, (1997).
7. Shahriari M.R., Ding J.Y., Wang C.M., Lin C.H., Sigel G.H. Sol-Gel and R.F. Sputtering Thin Film Coatings for Fiber Optic Sensor Applications. MRS proceedings. 244, (1991).
8. Krihak M.K., Shahriari M.R. A highly sensitive, all solid state fiber optic oxygen sensor based on the sol-gel coating technique. Electron. Lett 32: 240–242, (1996).
9. Dave B.C., Dunn B., Selverstone V.J., Zink J.I. Sol-gel encapsulation methods for biosensors. Anal. Chem. 66: 1120A–1127A, (1994).
10. Van Emon J.M., and Gerlach C.L. Bioseparation and bioanalytical techniques in environmental monitoring. Anal chim. Acta, 376: 55, (1998).
11. Rodriguez-Mozaz S., Lope´z de Alda M. J. and Barcelo D. Biosensors as useful tools for environmental analysis and monitoring, Anal. Bioanal. Chem. 386(4): 1025–1041, (2006a).
12. Paula C.A., Jer´onimo A.N., Ara´ujo M.C., Montenegro B.S.M. Optical sensors and biosensors based on sol-gel films. Talanta 72: 13-27, (2007).
13. Dickey F.H. Specific adsorption. J. Phys. Chem. 58: 695-707, (1955).
14. Avnir D., Braun S., Lev O., Levy D., Ottolenghi M. Enzymes and Other Proteins Entrapped in Sol-Gel Materials. Chem. Mater. 6:1605-1614, (1994).
15. Larry L.H., Jon K.W. The sol-gel process. Chem. Rev. 90: 33–72, (1990).
16. Shtelzer S., Rappoport S., Avnir D., Ottolenghi M., and Braun S. Properties of trypsin and of acid phosphatase immobilized in sol-gel glass matrices. Biotechnol. Appl. Biochem 15:227-235, (1992).
17. Gvishi R., Ruland G., and Prasad P.N. New laser medium: dye-doped sol-gel fiber. Optics Commun, 126: 66-72, (1996).
18. Klein L.C. (Ed.), Sol–gel Optics: Processing and Applications, Kluwer Academic, Boston, MA, 345, (1994).
19. Lin J., Brown C.W. Sol-gel glass as a matrix for chemical and biochemical sensing, Trends Anal. Chem, 16: 200-207, (1997).
20. Wang J., Park D.S., Pamidi P.V.A., Tailoring the Macroporosity and Performance of Sol-Gel derived Carboncomposite Glucose Sensors, J. Electroanal. Chem, 434: 185-191, (1997).
21. Brinker C.J., Scherer G.W. (Ed.), Sol–Gel Science: The physics and chemistry of sol-gel processing, Academic Press, San Diego, CA, (1990).
22. Mennig M.M., Zahnhaussen H. Schmidt, Proc. Novel nonhydrolytic sol-gel route to low –OH- and CH- containing organic-inorganic composites. SPIE, 3469: 68 (1998).
23. Duhua Wang., Gordon P Bierwagen. Sol–gel coatings on metals for corrosion protection. Progress in Organic Coatings 64, 327–338, (2009).
24. Barbé C.J., Cassidy D.J. Sol-Gel Bonding Wafers Part 1: Influence of the Processing temperature on final bond morphology and Interfacial energy. Thin Solid Films 488: 153-159, (2005).
25. Zusman R., Rottman C., Ottolenghi M., and Avnir D. Doped Sol-Gel Glasses as Chemical Sensors. J Non cryst. Solids, 122: 107-109, (1990).
26. Rottman C., Ottolenghi M., Zusman R., and Lev O. Doped Sol-Gel Glasses as pH Sensors. Mat. Letters 13: 293-298, (1992).
27. Chaudhury N.K., Bhardwaj R., and Murari B. M. Fluorescence spectroscopic study to characterize and monitor TEOS based sol–gel process for development of optical biosensors. Current Applied Physics, 177-184, (2003).
28. Gupta R., Mozumdar S., Chaudhury N.K. Fluorescence spectroscopic studies to characterize the internal environment of tetraethyl-orthosilicate derived sol–gel bulk and thin films with aging. Biosensors and Bioelectronics. 1358-1365, (2005).
29. Bhaskar M.M., Anand S., Nivedita K.G., Chaudhury N.K. Fluorescence spectroscopic study of dip coated sol-gel thin film internal environment using fluorescent probes Hoechst33258 and Pyranine. J Sol-Gel Sci Tech.41: 147–155, (2007) .
30. Dunn B., Miller J.M., Dave B.C., Valentine J.S., Zink J.I. Strategies for encapsulating biomolecules in sol-gel matrices. Acta. Mater; 46 (3): 737-741, (1998).
31. Kim D.W., Oh S.G., Yi S.C., Bae S.Y., and Moon S.K. Preparation of Indium−Tin Oxide Particles in Shear-Induced Multilamellar Vesicles (Spherulites) as Chemical Reactors Chem. Mater, 12:996-1002, (2000).
32. Wang B., Zhang J., Dong S., Silica sol–gel composite film as an encapsulation matrix for the construction of an amperometric tyrosinase-based biosensor. Biosens Bioelectron, 15(7-8):397-402 (2000).
33. Liu C.Y., and J M Hu. Hydrogen peroxide biosensor based on the direct electrochemistry of myoglobin immobilized on silver nanoparticles doped carbon nanotubes film. Biosensors and Bioelectronics 24, 2149-2154, (2009).
34. Jonathan F, Jing C, Zhou E, Lan H, Bruce D. Jeffrey I Z. Bio-hybrid materials for immunoassay-based sensing of cortisol. J Sol-Gel Sci. 50,176-183, (2009).
35. Sumio sakka. Preparation and properties of Sol-gel coating films. Journal of Sol-Gel Science and Technology. 2, 451-455, (1994).
36. Monika L, Iwona H, Agnieszka U, Halina P. Optical properties of sol-gel coating for fiber-optic sensors. Surface Coating Tech. 151-152, 299-302, (2002).
37. Mercedes Perullini, Matı´as Jobba´gy, Sara A. Bilmes, Iris L. Torriani, Roberto Candal. Effect of synthesis conditions on the microstructure of TEOS derived silica hydrogels synthesized by the

alcohol-free sol–gel route. Journal of Sol-Gel Science and Technology 59:174–180, (2011).
38. Brinker C.J, Frye G.C, Hurd A.J, Ashley C.S, Fundamentals of sol-gel dip coating. Thin solid films, 201(1), 97–108, (1991).
39. Schröder H. Physics of Thin Films. Academic Press, New York - London, 5, 87-141, (1969).
40. Klein L.C (Ed.), Sol-Gel Technology for Thin Films, Fibers, Preforms, Electronics, and Specialty Shapes, Noyes Publications, Park Ridge, 407, (1988).
41. Juan L, Swee Ngin T, Hailin G. Silica sol-gel immobilized amperometric biosensor for hydrogen peroxide. Analytica Chimica Acta 335, 137-145, (1996).
42. Jeffrey D, Jordan, Richard A, Bright F.V. Aerosol-generated sol-gel-derived thin films as biosensing platforms. Analytica Chimica Acta, 332, 83-91, (1996).
43. McDonagh C, Sheridan F, Butler T, MacCraith B.D. Characterisation of sol-gel-derived silica films. Journal of Non-Crystalline Solids, 194, 72-77, (1996).
44. Xie H, Wei J, Zhang S, Zhao L. Ccharacterization of Sol-gel by spectroscopic Ellipsometry. Journal of Physics, 28, 95-99, (2006).
45. C. McDonagh, P. Bowe, K. Mongey, B.D. MacCraith. Characterization of porosity and sensor response times of sol–gel-derived thin films for oxygen sensor applications. Journal of Non-Crystalline Solids, 306, 138–148, (2002).
46. Lalonde J., Margolin A. Enzyme Catalysis in Organic Synthesis. Wiley-VCH, Weinheim, 163-185, (2002).
47. Chibata I. Immobilized Enzymes. John Wiley, New York, 1-73, (1978).
48. Murty V.R, Bhat J, Muniswaran P.K.A. Hydrolysis of oils by using Immobilized lipase enzyme: a review. Biotechnol. Bioprocess Eng., 7, 57-66, (2002).
49. Kennedy J. F & Cabral J. M. S. Enzyme Immobilization. Biotechnology, 7a, 347-404, (1987).
50. Khan A. A & Alzohairy M. A. Recent Advances and applications of immobilized enzyme technologies. Res. J. Biol. Sci., 5(8), 565-575, (2010).
51. Sheldon R. Enzyme Immobilization: The quest for optimum performance. Adv. Synth. Catal, 349, 1289–1307, (2007).
52. Kandy L, Rounsaville J. F, Schutz G. Ullmann's Encyclopedia of Industrial Chemistry. Ed. Vol, A14, Ed. VCH, Weinheim, 1-48, (1987).
53. Taylor R.F. Protein Immobilization: Fundamentals and Applications. New York Chap. 8, 263-303, (1991).
54. Ingersoll C.M, Bright F.V, Using sol-gel based platform for chemical sensors, Chemtech. 27, 26-31, (1997).
55. Wang J, Pamidi P.V.A, Park D.S, Screen-printable sol-gel enzyme-containing carbon inks, Anal. Chem. 68, 2705-2708, (1996).
56. Gill I, Ballesteros A, Bioencapsulation within synthetic polymers (part 1): sol-gel encapsulated biological, TIBTECH 18, 282-296, (2000).
57. Gill I, Bio-doped Nanocomposite Polymers: Sol−Gel Bioencapsulates, Chem. Mater. 13, 3404-3421, (2001).
58. Ying Liu, Zimple Matharu, Michael C. Howland, Alexander Revzin, Aleksandr L. Simonian, Affinity and enzyme-based biosensors: recent advances and emerging applications in cell analysis and point-of-care testing. Anal Bioanal Chem, 404(4):1181-96, (2012).
59. Andreou V.G, Clonis Y.D, A portable fiber-optic pesticide biosensor based on immobilized cholinesterase and sol-gel entrapped bromcresol purple for in-field use, Bios. Bioelectron. 17, 61-69, (2002).
60. Wang K.M, Li J, Yang X, Shen F, Wang X, A chemiluminescent H_2O_2 sensor based on horseradish peroxidase immobilized by sol-gel method, Sens. Actuators B: Chem. 65, 239–240., (2000).
61. Narang U, Prasad P.N, Bright F.V, Ramanathan K, Kumar N.D, Malhotra B.D, Kamalasanan M.N, Chandra S, Glucose Biosensor Based on a Sol-Gel-Derived Platform, Anal. Chem. 66, 3139-3144, (1994).
62. Ferretti S, Lee S.K, MacCraith B.D, Oliva A.G, Richardson D.J, Russell D.A, Sapsford K.E, Vidal M, Optical Biosensing of nitrite ions using cytochrome cd1 nitrite reductase encapsulated in a sol-gel matrix, Analyst 125 (11), 1993-1999, (2000).
63. Premkumar J.R, Rosen R, Belkin S, Lev O, Sol-gel luminescence biosensors: Encapsulation of recombinant E Coli reporters in thick silicate films, Anal. Chim. Acta 462, 11-23, (2002).
64. Glezer V, Lev O, Sol-gel vanadium pentaoxide glucose biosensor, J. Amer. Chem. Soc. 115, 2533- 2534, (1993)
65. Pankratov I, Lev O, Sol-gel derived renewable-surface biosensors, Journal of Electro analytical Chemistry 393, 35-41, (1995).
66. Roux C, Livage J, Farhati K and Monjour L, Antibody-Antigen Reactions in Porous Sol-Gel Matrices, J. Sol-gel science & Tech. 8, 663-666, (1997).
67. J. Li, L. S. Chia, N. K. Goh, and S. N. Tan, "Silica sol-gel immobilized amperometric biosensor for the determination of phenolic compounds," Analytica Chimica Acta, 362, 2-3, 203–211, (1998).
68. Toshio Y & Kazuyoshi T, Amperometric biosensor with a composite membrane of sol-gel derived enzyme film and electrochemically generated poly (1,2-diaminobenzene) film, Biosensors and Bioelectronics, 13(1), 67-73, (1998).

69. Yang S, Lu Y, Atanassov P, Wilkins E, Long X, Microfabricated Glucose Biosensor with Glucose-Oxidase entrapped in Sol-Gel Matrix, Talanta, 47, 735−743, (1998).
70. Otto S.W, Ines O, Natalya P, Klimant I, Sol-gel based glucose biosensors employing optical oxygen transducers, and a method for compensating for variable oxygen background, Biosensors & Bioelectronics, 15, 69–76, (2000).
71. Kumar A, Malhotra R, Malhotra B. D, Grover S. K, Tetraethylorthosilicate film modified with protein to fabricate cholesterol biosensor, Anal. Chim. Acta. 414, 43-50, (2000).
72. Pulido-Tofiño P, Barrero-Moreno J.M, Pérez-Conde M.C, Sol–gel glass doped with isoproturon antibody as selective support for the development of a flow-through fluoro- immunosensor, 429, 337-345, (2001).
73. Audebert P, Demaille C, Sanchez C, Electrochemical probing of the activity of glucose oxidase embedded sol-gel matrixes, Chem. Mater. 5 (7), 911–913, (1993).
74. Ramos M. C, Torijas M. C, Navas D. A, Enhanced chemiluminescence biosensor for the determination of phenolic compounds and hydrogen peroxide, Sens. Actuators, B, 73, 71-75, (2001).
75. Kim A, Lee W.Y, Amperometric phenol biosensor based on sol–gel silicate/Nafion composite film, 120-749, (2002).
76. Shi Q, Peng T, Zhu Y, Catherine F. Y, An Electrochemical Biosensor with Cholesterol Oxidase / Sol-Gel Film on a Nanoplatinum/Carbon Nanotube Electrode, Electroanalysis, 17(10):857 – 861, (2005).
77. Jer´onimo P.C.A, Ara´ujo A.N, Montenegro M.C.B.S.M, Satinsk´y D, Solich P, Flow-through sol-gel optical biosensor for the colorimetric determination of acetazolamide, Analyst 130(8), 1190-1198, (2005).
78. Nguyen-Ngoc H, Tran-Minh C, Fluorescent biosensor using whole cells in an inorganic translucent matrix, Analytica Chimica Acta 583, 161-165, (2007).
79. Ramanathan M, Luckarift H.R, Sarsenova A, Wild J.R, Ramanculov E.K, Olsen E.V, Simonian A.L, Lysozyme-mediated formation of protein-silica nano-composites for biosensing applications, Colloids Surf B Biointerfaces 73, 58–64, (2009).
80. Verma N, Kumar R, Sachin M, Simple, qualitative cum quantitative, user friendly biosensor for analysis of Urea, Advances in Applied Science Research, 3 (1):135-141, (2012).
81. Kim M.A, Lee W.Y, Amperometric phenol biosensor based on sol-gel silicate/Nafion composite film, Analytica Chimica Acta 479, 143-150, (2003).
82. Miao, Y., Tan, S.N. Amperometric hydrogen peroxide biosensor with silica sol–gel/chitosan film as immobilization matrix, Analytica Chimica Acta 437, 87–93, (2001).
83. Xuecai, T, Minjian, L, Peixiang C, Lijun L, Xiaoyong Z. An amperometric cholesterol biosensor based on multiwalled carbon nanotubes and organically modified sol-gel/chitosan hybrid composite film. Anal. Biochemistry 337, 111–120, (2005).
84. Shustak G, Marx S, Turyan I, Mandler D. Application of sol-gel technology for electroanalytical sensing. Electro analysis 15, 5-7, (2003).
85. Flora K.K, Tucker K.T, Hogue C.W, Brennan J.D. Screening of antagonists based on induced dissociation of a calmodulin-melittin interaction entrapped in a sol-gel derived matrix. Anal. Chim. Acta 470 (1), 19-28, (2002).
86. Quanlin W, Gongxuan L, Baojun Y. Hydrogen peroxide biosensor based on direct electrochemistry of hemoglobin immobilized on carbon paste electrode by a silica sol–gel film. Sensors and Actuators B 99, 50-57, (2004).
87. An Doong R, Hsiao-Chung T. Immobilization and characterization of sol-gel encapsulated acetylcholinesterase fiber optic biosensor. Analytica Chimica Acta 434, 239–246, (2001).
88. Li J, Chia L.S, Goh N.K, Tan S.N, and Ge H. Mediated amperometric glucose sensor modified by the sol-gel method. Sensors Actuators. B, 40, 135-141, (1997).
89. Saipeng Y, Lu Y, Plamen A, Ebtisam W, Xiangchun L. Microfabricated glucose biosensor with glucose oxidase entrapped in sol-gel matrix. Talanta, 47(3), 735-743, (1998).
90. Narang U, Prasad P.N, Bright F.V, Ramanathan K, Kumar N.D, Malhotra B.D, Kamalasanan M.N, Chandra S. A novel protocol to entrap active urease in a tetraethoxy-silane derived sol-gel thin-film architecture. Chem. Mater. 6, 1596-1598, (1994).
91. Andreou V.G, Clonis Y.D. Novel fiber-optic biosensor based on immobilized glutathione S-transferase and sol-gel entrapped bromcresol green for the determination of atrazine. Anal. Chim. Acta., 460, 151-161, (2002).
92. Won-Y.L, Seung-Ryeol, K, Tae-Han K, Kang S. L, Min-Chol S, Je-Kyun P. Sol–gel-derived thick-film conductometric biosensor for urea determination in serum. Analytica Chimica Acta 404, 195–203, (2000).
93. Xu C, Shaojun D. Sol-gel derived titanium oxide/ copolymer based glucose biosensor. Biosensors and Bioelectronics 18, 999 – 1004, (2003).
94. Alisa B, Nadav A, David A, Turniansky A and Miriam A. Sol-gel matrices doped with Atrazine antibodies: Atrazine binding properties. Chem. Mater. 9, 2632-2639, (1997).
95. Lan E. H, Dunn B and Zink J.I. Sol-Gel Encapsulated Anti Trinitrotoluene (TNT) Antibodies in immunoassays for TNT. Chem. Mater. 12, 1874-1878, (2000).
96. Moran S and Altstein M. Sol-Gel Entrapped Levonorgestrel Antibodies: Activity and Structural

Changes as a Function of Different Polymer Formats. Materials 4, 469-486, (2011).
97. Tan F, Yan F, Huangxian J. Sensitive reagentless electrochemical immunosensor based on an ormosil sol–gel membrane for human chorionic gonadotrophin. Biosensors and Bioelectronics 22, 2945–2951, (2000).
98. Wu J, Feng Y, Xiaoqing Z, Yuetian Y, Tang J and Huangxian J. Disposable Reagentless Electrochemical Immunosensor Array Based on a Biopolymer/Sol-Gel Membrane for Simultaneous Measurement of Several Tumor Markers. Clinical Chemistry 54, 1481–1488, (2008).
99. Jing C. Zhou, Maria H. Chuang, Esther H. Lan, Bruce Dunn, Patricia L. Gillman and Scott M. Smith. Immunoassays for cortisol using antibody-doped sol–gel silica. J. Mater. Chem., 14, 2311-2316, (2004).
100. Moran S, Miriam A. Sol-Gel Entrapped Levonorgestrel Antibodies: Activity and Structural Changes as a Function of Different Polymer Formats. Materials, 4(3), 469-486, (2011).
101. H G. Wankhade*, S. V. Manorama and G.N.Chaudhari. Synthesis and Characterization of Peroxide Sensor based on PVA-PANI nanocomposite. Int J Pharm Bio Sci; 6(2): 422 – 429, (2015).