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Role of nanostructured networks as analytical tools for biological systems

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1. ABSTRACT

In recent years, nanostructured materials have emerged as potential candidates offering excellent prospects for interfacing the detection of biomolecules. Nanomaterials such as nanoparticles, nanostructured silicates, nano-sized metal oxides, nanostructured polymers, quantum dots, nanocomposites and sensing nanodevices are being utilized worldwide for fabrication of chemical sensors and sensor arrays with tailored characteristics and tuneable properties. Among above, the materials that create a matrix structure at the nanoscale level are particularly fascinating. The exceptional physical, chemical, mechanical and electrical properties of these matrices advocate their application in the electrode modification resulting in sensing devices and transducers with superior performance. Here we present an overview of different types of nanostructured networks that are applied in sensor development. The role of these materials in chemical sensors is described along with the techniques that are the backbone of the sensing process. Special attention has been given to some key sensors that are directly related to human physiology and have clinical significance.

2. INTRODUCTION

The potential of nanostructured domain is increasingly recognized by the scientific society and the technological world. The same is corroborated with exponential rise in the number of research publications in this area. In addition, across the globe the biological, physical, chemical, engineering and materials science communities and government bodies have been organizing workshops, meetings and conferences around various aspects of research in nanoscience with increasing frequency. The exciting properties of nanomaterials present these systems as components of optical (1), electrical (2-4), electrochemical (5,6) and catalytic sensors and devices (7,8).

Nanomaterials can be broadly classified into nanostructured and nanophase/nanoparticle materials. The former refer to condensed bulk materials made up of grains with grain sizes in the nanometer range, whereas the latter are usually the dispersive nanoparticles. The nanometer size here covers a wide range which can be as large as 100-200 nm. Nanoparticles of noble metals, polymers, metal oxides etc. are of immense interest due to their chemical,

electrical and optical properties (9-13). For application point of view, dispersion of these nanoparticles in solid state matrices (14-16) is one of the premier requirements, while avoiding or controlling aggregation phenomena. But in reality, in solid matrix, these nanoparticles often agglomerate into larger irregular structures and seldom have a uniform dispersion. Also, a variety of reducing agents and/or stabilizers that are used for preparation and stabilization of nanoparticles (17-21) affect their solid state properties.

On the other hand, the nano-sized networks of polymers, ceramic materials or metal oxides along with some of the composites provide with solid state matrices, exciting inherent properties having potential for diverse applications (22-26). In addition, these matrices also provide a means for entrapment of conventional nano-sized entities such as metal nanoparticles (27-29), carbon nanotubes (30-31), nanoclay (32-34) etc. while maintaining their size-specific properties intact. There is a lot of research work undergoing on these nanostructured networks (35-38) at present. Still, no recent overview has appeared, however, to the best of our knowledge that focuses especially upon the significance of the nanostructured networks resulting from above matrices with special emphasis on their application for bioanalytical purposes. Hence, the purpose of the present review is to fill this gap by summarizing current accomplishments in preparing and characterizing nanostructured films of different materials and to point out their potential applications in the direction of electroanalytical chemistry of biological systems.

In selecting the topics for this thematic issue of encyclopedia, we have put emphasis on the application of novel nanostructured materials and their integration into chemical sensors for bioanalytical purposes (22-25). The field of chemical sensors, while rooted in chemistry, is highly interdisciplinary; bridging between physics, chemistry, biology, engineering and medical sciences. Molecular recognition, materials science and chemical and physical transduction are all essential aspects of the field that draw upon the traditional areas of chemistry, including inorganic, organic, physical, analytical and biological sciences. Fully integrated sensing systems adopt numerous other technologies and sciences. Thus, here an attempt is made to summarize the current accomplishments and future prospects in this intellectually fascinating and highly relevant area of current research. The currently undergoing activities would soon become “past achievements” and concerned readers would need to acquaint themselves with the latest results as they appear in premier publications and/or circulated at scientific gatherings.

3. TYPES OF NANOSTRUCTURED NETWORKS

The nanostructured networks listed below are the ones that are primarily utilized in the development of chemical and biological sensors. Manipulation of the novel properties of these materials for fabrication of sensor devices is discussed in following sections.

3.1. Organically modified silicates

The sol-gel technology, which is a type of solution based chemistry, creates inorganic networks through the formation of a colloidal suspension in a liquid (sol). The hydrolysis, condensation and subsequent gelation of the same lead to the formation of a dense network in a continuous liquid phase (gel). Precursors for creating these colloids are alkoxides (39,40) and alkoxysilane derivatives (41,42) surrounded by various reactive ligands imparting desired functionalities (43-45). The sol-gel process allows the fabrication of a wide range of materials (46-48) viz. ultra-fine powders, monolithic ceramics and glasses, ceramic fibers, inorganic membranes, thin film coatings and aerogels. These materials offer several advantages over organic polymeric materials including physical rigidity and higher abrasion resistivity, negligible swelling in both aqueous and non-aqueous solvents, chemical inertness (49), high bio-degradational (50-52) and chemical stability, excellent optical transparency and low intrinsic fluorescence. These properties of sol–gel glass materials accommodate diverse sensing requirements (53-55) and convert such design at commercial scale. The introduction of organic modification (56-58) in sol–gel matrices have been used to control the degree of cross linking to improve film adhesion to solid supports, reduce the concentration of surface silanol groups and the ion-exchange capacity and to introduce reactive functionalities that can be subsequently used for anchoring of molecular recognition species on prepared xerogels. Monomers containing a Si-C bond and easily derivatized radicals such as amino, vinyl, glycidoxy, mercapto and epoxy groups can be used to prepare readily derivatized xerogels and the subsequent sol-gel matrix can be referred as organically modified silicate (Ormosil) (59-61).

The ormosils provide a convenient way to synthesize a host matrix and are highly suitable for microencapsulation (62,63). Gels, which are dispersions of colloidal particles, are composed of an interconnected, rigid network with pores of nano-size dimensions and polymeric chains with whole average length not greater than a micrometer. The term “gel” embraces a diversity of combinations of substances that can be classified into four categories: (i) well-ordered lamellar structures, (ii) completely disordered covalent polymeric networks, (iii) predominately disordered polymer networks formed through physical aggregation and (iv) particular disordered structures. The initial hydrolysis and polycondensation reactions in a localized region lead to the formation of colloidal particles. As the interconnection between these particles increases, the viscosity of sol starts to increase and leads to formation of a solid gel. Because protons or hydroxide ions are required for catalysis in silica gel formation, the pH of the reaction medium is also an important factor. Acid catalysts (64-66) increase the rate of hydrolysis and do not favor condensation reactions, whereas base catalyzed hydrolysis (67,68) produces rapid condensation. The biomolecule to be encapsulated can be added to the sol which apparently after cross linking and polycondensation, increases the viscosity of sol and subsequently solidifies. This process continues and a porous matrix is formed around the molecule, trapping it

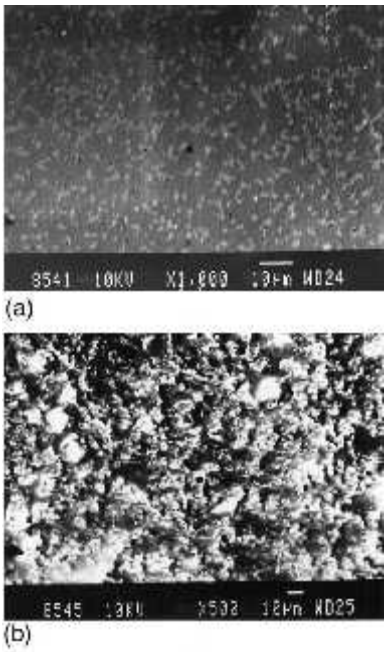

Figure 1. SEM of ormosil in the absence of enzyme (a) and in the presence of enzyme (b). Reproduced with permission from (69).

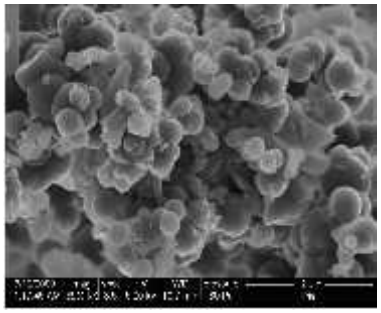

Figure 2. Chemically synthesized Polyindole nanospheres using ethanol-water emulsion. Reproduced with permission from (86).

inside. This process produces a bulk material in which the biomolecule is confined even though it has not reacted with or otherwise attached itself to the matrix. Figure 1 shows the SEM image of an ormosil film formed over the surface of Indium Tin Oxide (ITO) electrode using alkoxysilane precursors in absence (a) and in presence (b) of glucose oxidase (69).

Organic modification (56-58) in sol–gel precursors is used to reduce the degree of crosslinking, improve film adhesion to its support, reduce the concentration of surface silanol groups and the ion exchange capacity, alter partition coefficients, or introduce reactive functionalities that can be subsequently used for covalent attachment of molecular recognition species. Such precursors contain desired functional groups in polymerization mixture, which, after following the sol–gel process lead to the formation of organically modified sol–gel glass (ormosil). Ormosils can be tailored from commercially available Organo-functional alkoxysilanes. They are used in the development of sensors particularly for attaching the sensing material to the surface of physiochemical transducers. The encapsulation of enzyme within ormosil has shown high storage and operational stability. Table 1 enlists the different types of sensors developed using nanostructured sol-gel matrices.

3.2. Nanostructured polymers

Since the last decade, nanostructured polymers, both conducting and insulating ones, have played key role in the progress of nanotechnology (70-73). These materials are porous and have a high surface area. The conducting polymers show low energy optical transitions, low ionization potential, high electron affinities and considerable ionic and electronic conducting properties (74-76). In addition, polymers can be easily synthesized, are comparatively inexpensive and can be functionalized using various patterning methods to achieve required optical, electronic or mechanical properties and they also demonstrate biocompatibility. These unique features lead to a variety of applications in analytical sciences, biosensor devices and drug release systems, as reviewed by various researchers (77-79).

These materials can be typically synthesized following addition and/or condensation polymerization techniques (80-85). Solution phase polymerization, emulsion, reverse emulsion and interfacial polymerizations are the common protocols that lead to the formation of polymers with desired morphologies. Figure 2 shows the SEM image of Polyindole formed using miscible solvent system of ethanol and water. Here, micelles are formed on the addition of indole monomer (dissolved in ethanol) to oxidizing agent (dissolved in water) (86). This results in the formation of a metastable organic-aqueous emulsion which acts as a template for polymer growth resulting in formation of spherical morphology. In addition, the conducting polymers can be synthesized in form of thin films following electrochemical polymerization (87,88) which offers a control over the film thickness and imparts excellent electrochemical properties. A number of conjugated polymers can be transformed from an insulating into a highly conductive state upon manipulation of polymer chain with specifically selected impurities, a process termed “doping”. While insulating polymers act primarily as an entrapment matrix in sensor fabrication, the conducting polymers also impart electrical conductivity and selectivity to the sensor. For this reason, the latter are much more explored in sensor development (23-26, 89-91). Table 2 enlists some of the sensors developed using nanostructured polymers.

Nanostructural control in polymeric systems involves the manipulation of polymer structure resulting in dimensions in the order of 1–100 nm. Novel materials are being fabricated and the possibilities of surface modification of conventional electrodes are explored, providing new and exciting properties which may be used in the development of sensing devices. Polymer chemists are working on development of necessary tools to control various molecular structural parameters in synthetic macromolecules, such as their molecular weight and polydispersity, regio- and stereo-regularity, topology of repeat unit connectivity, such as in the case of dendrimers

Table 1. List of some sensors based on organically modified silicates

Substrate	Redox mediator	Biomolecule	Analyte	Sensing Technique	Reference
Pt[1]/Sol-gel	FMCA[2]	GOD[3]	Glucose	Amperometry	53
Pt/Sol-gel	FMCA	Tyrosinase	Dopamine	Amperometry	54
ITO[4]/Sol-gel	PB[5]	-	H_2O_2	Amperometry	125
ITO/Sol-gel	PB	-	Dopamine	Amperometry	131
Pt/PAni[6]/Sol-gel	-	Creatininase, Creatinase Urease	Creatinine Urea	Potentiometry	156
Pt/Sol-gel	FMCA	GOD	Glucose	Amperometry	178
ITO/Sol gel	PB	-	H_2O_2	Amperometry	179
ITO/Sol-gel	Potassium Ferricyanide	-	Ascorbic acid H_2O_2	Amperometry	180
ITO/Sol-gel	FMCA	-	NADH Ascorbic acid	Amperometry	181
GC/Sol-gel	FMCA	-	NADH	Amperometry	182
Pt/Sol-gel	TTF-TCNQ[7]	GOD	Glucose	Amperometry	183

Abbreviations: Platinum[1], Ferrocene monocarboxylic acid[2], Glucose oxidase[3], Indium tin oxide[4], Prussian Blue[5], Polyanilne[6], Tetrathifulvalene-tetracyanoquinodimethane[7]

and to a limited extent up to copolymer sequence distribution. Numerous articles are available describing the application of polymeric nanomaterials in biosensing and immunosensor technology (89-91).

3.3. Nanocrystalline metal oxides

Nanostructured metal oxides represent a broad class of materials whose properties stretch from metals to semiconductors and even insulators. These materials are known for their excellent mechanical, chemical, physical, thermal, electrical, optical, magnetic and also surface (92-98) properties. Their application in the field of chemical sensing has originated from the simple and well known theory that the electrical conductivity of semiconductors varies with the composition of the gas atmosphere surrounding them. For this particular reason, metal oxides are primarily reported for fabrication of gas sensors. These nanocrystalline materials can be classified into *zero dimensional* for nano-sized clusters, *mono dimensional* for nanowires and *two dimensional* for thin films depending upon the number of dimensions that are nano-sized.

Vapour phase deposition (99,100) is the most commonly adopted route for synthesis of nanocrystalline metal oxides. Other methods are thermochemical and sonochemical routes (101). For the past few years research in the direction of nanocrystalline metal oxides has directed worldwide scientific community to purport metal oxide sensors which present the same as nanostructures, nanoelectronics, nanobiomaterials, nanobioactivators, etc. (102,103). In the last decade a large variety of nanostructured metal oxide (ZnO, TiO_2, ZrO_2, SnO_2, CeO_2, MnO_2) based devices with new capabilities have been reported (92-96). Figure 3 shows the TEM image of chemically synthesized TiO_2 after calcination at 900 ^{0}C (104). The semiconducting, piezoelectric and pyroelectric properties of these nanostructured metal oxides find interesting applications in optoelectronics and catalysis. These materials have wide band gaps and higher binding energy (e.g. ZnO = 60 meV) and are optically transparent and reflective, thus making them ideal candidates for fabrication of ultraviolet light-emitting diodes and lasers. These nanostructures exhibit high surface area, nontoxicity, good bio-compatibility, high isoelectric point (IEP) and chemical stability. In addition, they also show biomimetic and excellent electron transport features, thus, imparting them great potential in sensor/biosensor fabrication. A number of reports have appeared in the literature on the application of nanostructured metal oxides for construction of immunosensors. Table 3 depicts different types of sensors developed using nanostructured metal oxides.

3.4. Composite nanostructures

Composite nanostructures, more commonly known as nanocomposites are composite materials in which at least one of the component phases has dimensions of the nanoscale (105-109). These materials, while presenting challenges related to the control of nanodimension in composite form, have emerged as suitable alternatives to overcome limitations of commonly used composite materials. Among all the nanomaterials, these are the ones that are studied the most across the globe. The number of research publications related to these materials has increased tremendously over the past few years. These materials are popularly called the materials of 21st century in the light of possessing design uniqueness and property combinations absent in conventional composites.

The nanocomposite materials can be classified based on their matrix materials, in three different categories viz. ceramic matrix nanocomposites, metal matrix nanocomposites and polymer matrix nanocomposites. Typical protocols for synthesis of nanocomposites are co-precipitation (110,111), template assisted synthesis (112), chemical vapour deposition (113,114) and electrochemical deposition (115,116). These involve synthesis of nanostructure either by *in situ* or *ex situ* methods. The former describe the protocols where all the components of the desired composite are mixed together. The complete interaction results into the formation of nano-sized composite materials. On the other hand, in *ex situ* approach, one of the components may act as a matrix and the other one (having nano-sized dimensions) acts as filler. The matrix provides a template for the interaction of the two components. In result, these heterogeneous nanocomposites exhibit properties that are quite different from their individual parent materials. Figure 4 shows the TEM image of Pd-TiO_2-SiO_2 nanocomposite after calcination at 900 ^{0}C (104). The presence of silica results in the formation of a well-ordered spherical geometry.

Table 2. List of some sensors based on nanostructured polymers

Substrate	Redox mediator	Biomolecule	Analyte	Sensing Technique	Reference
Si[1]/BDD[2]/PAni	-	GOD	Glucose	Amperometry	23
Pt/PAN[3]	-	AChE[4]	Acetylcholine	Amperometry	24
GCE[5]/PEI[6]	-	-	NADH	Amperometry	25
GCE/PI6CA[7]	-	ssDNA[8]	DNA	Amperometry	26
ITO/PAni	-	ssDNA[9]	DNA	Amperometry	128
Au[10]/PVA[11]	-	GOD	Glucose	Amperometry	151
GCE/ABSA[12]	-	HRP[13]	H_2O_2	Amperometry	155
Au/MIPM[14]	Potassium ferricyanide	-	Glucose	Amperometry	179
ITO/PAni	-	-	Dopamine Ascorbic acid	Amperometry	186
ITO/ PVdF[15]/PAPBA[16]/ NFM[17]	-	-	Glucose	Amperometry	187

Abbreviations: Silicon[1], Boron doped diamond[2], Acrylonitrile-methylmethacrylate-sodium vinylsulfonate[3], Acetylcholinesterase[4], Glassy carbon electrode5, Polyethyleneimine[6], Poly (indole-6-carboxylic acid)[7], single stranded DNA from Hepatitis B virus[8], single stranded DNA from *Neisseria gonorrhoeae*[9], Gold[10], Polyvinyl alcohol[11], *p*-aminobenzene sulfonic acid[12], Horseradish peroxidase[13], Molecularly imprinted polymer membrane[14], Poly(vinylidene fluoride)[15], Poly(aminophenylboronic acid)[16], Nanofibrous membrane[17]

Table 3. List of some sensors based on nanocrystalline metal oxides

Substrate	Biomolecule	Analyte	Sensing Technique	Reference
ITO/ZnO[1]	Urease GLDH[2]	Urea	Amperometry	92
ITO/ZnO	ChOx[3]	Cholesterol	Amperometry	93
Ag/ZnO	-	Ca^{2+}	Potentiometry	94
ITO/ZnO	ssDNA	DNA	Amperometry	145
ITO/CeO_2[4]	Lipase	Triglyceride	Amperometry	146
ITO/ZnO	GOD	Glucose	Amperometry	152
Si /TiO_2[5]	GOD	Glucose	Amperometry	153
Au/CeO_2	GOD	Glucose	Amperometry	154
Ag/ZnO	GOD	Glucose	Potentiometry	157
Si/ SiO_2[6]	GOD	Glucose	Potentiometry	158
ISFET[7]/SiO_2	GOD	Glucose	Potentiometry	159
Porous Alumina/SnO_2[8]	GOD	Glucose	Conductometry	160

Abbreviations: Zinc Oxide[1], Glutamate dehydrogenase[2], Cholesterol oxidase[3], Ceric oxide[4], Titanium oxide[5], Silicon oxide[6], Ion selective field effective transistor[7], Stannous oxide[8]

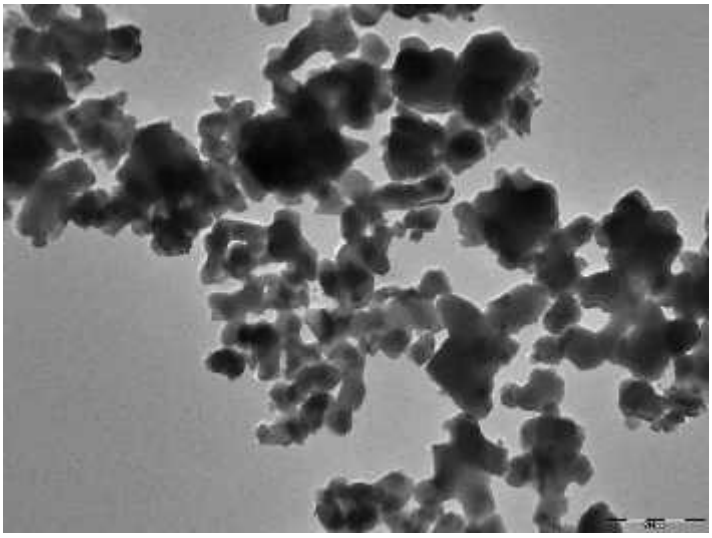

Figure 3. TEM image of nano-sized TiO_2 calcinated at 900 ^{0}C. Reproduced with permission from (104).

The nanocomposites combine the optical, electrochemical and catalytic properties of the inorganic metal nanoparticles and the electrical properties of the polymers, metal oxides and silicates which greatly widen their applicability in the field of sensors (117-119). These materials are hybrids of organic and inorganic molecules. Structural flexibility, ease of processing, tuneable electronic properties and potential for semiconducting behavior are characteristic of organic components, whereas inorganic constituents provide improved carrier mobilities, band gap tenability, dielectric properties and mechanical stability. The high electron shuttling features, large surface

Table 4. List of some sensors based on nanostructured composites

Substrate	Redox mediator	Biomolecule	Analyte	Sensing Technique	Reference
GCE/NiO[1]/AgNF[2]	-	-	Glucose	Amperometry	22
GCE/PSi[3]	FcA[4]	GOD	Glucose	Amperometry	117
GCE/PAni/AuNp[5]	-	-	Dopamine	Amperometry	118
GECE[6]/PSMNP[7]	-	-	H_2O_2	Amperometry	119
CPE[8]/Sol-gel	FMCA	GOD	Glucose	Amperometry	130
CPE/Sol-gel	Potassium Ferricyanide	HRP	H_2O_2	Amperometry	132
GCE/PEDOT[9]/AuNp	-	-	Dopamine Uric acid Ascorbic acid	DPV	133
GCE/PPy[10]/SWNT[11]	-	HRP	H_2O_2	Amperometry	134
GCE/PAni/AuNp	-	GOD	Glucose	Amperometry	135
GCE/MnO_2[12]/GO[13]	-	-	H_2O_2	Amperometry	147
GCE/PPy/Pt	-	-	H_2O_2	Amperometry	148
CPE/Sol-gel	TTF TCNQ dmFc[14]	ChO[15] AChE	Choline Acetylthiocholine	Amperometry	150
CPE/Sol-gel	FMCA	HRP	H_2O_2	Amperometry	184
Si/ZnO/PVP[16]	-	-	H_2O_2	Conductometry	185

Abbreviations: Nickel oxide[1], Silver nanofibres[2], Polysiloxane[3], Ferrocenealdehyde[4], Gold nanoparticles[5], Graphite epoxy composite electrode[6], Polymer stabilized metal nanoparticles[7], Carbon paste electrode[8], Poly(3,4-ethylenedioxythiophene)[9], Polypyrrole[10], Single walled carbon nanotubes[11], Manganese oxide[12], Graphene oxide[13], Dimethyl ferrocene[14], Choline oxidase[15], Poly(vinyl pyrrolidone)[16]

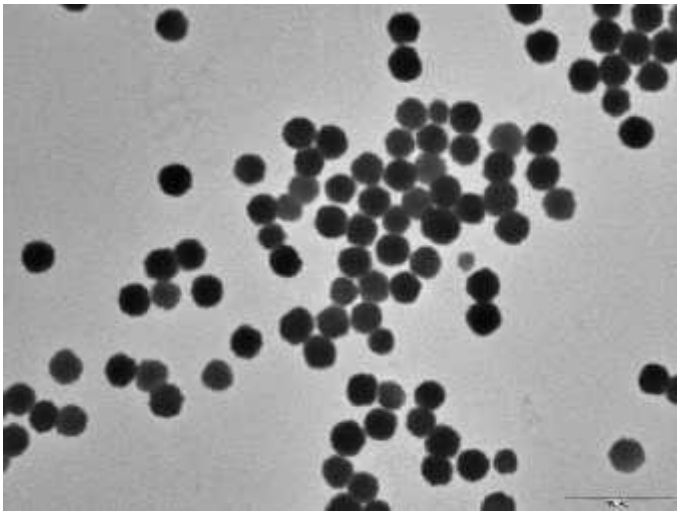

Figure 4. TEM image of nano-sized Pd-TiO_2-SiO_2 nanocomposite calcinated at 900^0C. Reproduced with permission from (104)

area, optical transparency and enhanced binding energies at organic-inorganic interfaces have been exploited in analytical sciences for sensor devices (117-119)). Table 4 shows some of the sensors developed using nanostructured composite matrices.

4. PROPERTIES OF NANOSTRUCTURED NETWORKS FOR APPLICATION IN SENSOR DEVICES

For the fabrication of an efficient sensor device, the choice of substrate for dispersing the sensing material significantly affects the sensor performance. In this regard, the materials having nanoscale dimensions exhibit distinctive properties (49-52,77-79,92-99) and offer prospects for tailoring physical, chemical and biological properties. In this section, we will discuss the main characteristics of nanostructured materials, which play key role in the fabrication of efficient sensing devices.

4.1. Effect of size

The nanoscale size has a direct effect (120-122) on the structural and chemical properties of nanostructures. Due to smaller size, the electron transport is significantly enhanced over the surface of these nanostructures. The nanoscale transducers reduce the pathway for direct electronic communication between redox biomolecule to the electrode for sensitive and faster analytic detection without posing hindrance.

4.2. Surface area

The large surface area (97,98) of nanostructured networks allows ease in the covalent attachment of specific biomolecules. It also provides stability and cross linking to impart the desired charge and solubility properties. Large surface-to-volume ratio of nanomaterials can enhance the surface energy of the surface atoms for attaching biomolecules. The reduced coordination number of the surface atoms increases the surface energy so that atom

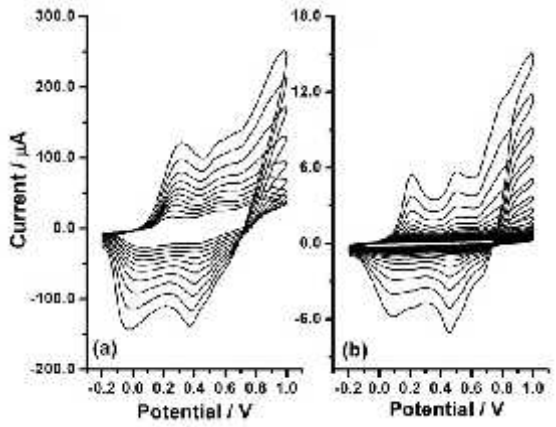

Figure 5. Potentiodynamic electropolymerization of 0.1 M aniline in 1.0 M HCl over (a) bare ITO electrode; (b) ormosil film deposited over ITO surface. Reproduced with permission from (129).

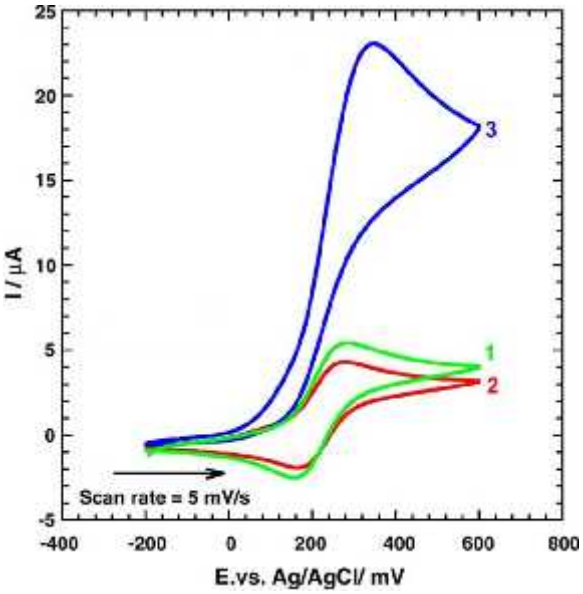

Figure 6. Cyclic voltammograms of ormosil film containing potassium ferricyanide before (1) and after the addition of ascorbic acid (2) followed by addition of dopamine (3). Reproduced with permission from (131).

diffusion occurs at relatively lower temperature. This is one of the primary requirements since for bioanalytical purposes the experimentation is required to take place at ambient temperature.

4.3. Porosity

The nanoscale pores (123,124) of these materials assist in the immobilization of electron transfer mediators (53-55), ion-carriers (125), enzymes (53,54), antigens (126,127), nucleic acids (26,128) and metal particles (54,125) on electrochemical transducer surfaces. In addition, the porous matrix also allows effective diffusion of analyte medium throughout the sensing matrix thereby facilitating the interaction of entrapped entities with analyte molecules. Thus, the matrix acts as a template for carrying out different chemical reactions and leads to amplification in the analytical signal of the biorecognition events. Figure 5 shows the cyclic voltammograms for the electropolymerization of Polyaniline (PAni) over (a) bare ITO electrode and (b) ormosil-modified ITO electrode (129). It can be observed that the number of cycles required for polymerization of aniline over bare ITO is very less as compared to that of ormosil modified ITO. However, the electrochemical behavior of PAni formed over ormosil-modified ITO is considerably better than in former case. This is due to the generation of a patterned nanostructure network resulting in controlled growth of PAni.

4.4. Biocompatibility

Biocompatibility of the sensing matrix is an important parameter in the development of competent sensing devices. The matrix must be able to retain the biological activity of immobilized biomolecules (53,54) after their attachment to the surface for longer duration of time in order to maintain the reusability and reproducibility of the sensor. The nanostructured matrix can entrap the biomolecules via electrostatic means (if it carries some oppositely charged particles) or through chemical route following covalent interaction or can even engulf the desired biomolecule during synthesis of the nanostructured network.

4.5. Catalytic activity

The nanostructured matrices can either perform the direct catalysis of target chemical reaction (117,118) or can immobilize external catalysts e.g. transition metal hexacyanometallates (125,128,130), metal particles, enzymes (53,54) etc. to carry out the catalytic reaction. The introduction of certain metal hexacyanometallates (which act as electron transfer mediators) and metal nanoparticles with catalytic properties into electrochemical sensors and biosensors can decrease the anodic or cathodic overpotentials of many analytically significant electrochemical reactions (117,118,125,128,130) without self-consumption of the same (*i.e.*, the catalyst undergoes several chemical transformations during the reaction, but is regenerated unchanged at the completion of the reaction). Figure 6 shows the cyclic voltammograms for the selective electrochemical determination of dopamine over ormosil film deposited over ITO surface (131). In this case, potassium ferricyanide is encapsulated in the ormosil film in order to introduce electrocatalysis to the sensing process.

4.6. Electrical conductivity

Some of the nanostructured networks exhibit significant electron transport properties. These are strongly dependent on the nanocrystalline structure of the matrix. In particular, conducting polymers, metal oxides and certain nanocomposites like PEDOT-Au nanocomposite, CNT-conducting polymer nanocomposite etc. are the most attractive materials due to their electrical conductivity, which can be monitored by recording the change in electrical conductivity of the transducing electrode (132-135). Electron transport properties of such nanomaterials are very important for electrical and electronic applications.

The high surface-to-volume ratio, chemical composition and crystal structure are important parameters that affect the electron transport mechanism. In addition, the electrical conductivity can be modulated via external means also by the addition of certain impurities (known as dopants). The conductivity is strongly influenced by binding of certain molecules as well.

5. ROLE OF NANOSTRUCTURED NETWORKS IN SENSOR DEVELOPMENT

The purpose of a chemical sensor is to provide real-time fast, selective and reproducible information about its surrounding chemical environment without perturbing it. One of the most important advantages of the use of tubular and other porous nanostructures in chemical sensors is the resultant increase in the quantity and activity of the immobilized biocatalysts (both useful to increase the sensitivity and stability of the resulting sensors). Considering their unique chemical-physical properties, in particular the high surface to volume ratio, nanostructured networks provide interesting opportunities for development of novel designs of biological sensors. These nano-sized matrices include organically modified silicates (Ormosils), nanostructured polymers, nanocrystalline metal oxides and composite nanostructures. These materials have the potential to be adopted and integrated into biomedical devices, since most biological systems including protein complexes, membranes and microbes etc. exhibit natural nano-sized dimensions. Currently, medicine and biomedical engineering are among the most promising and challenging fields involved in the application of nanostructured materials (53,54,125). Rapid advancements of nanostructured materials have been made in a wide variety of biomedical applications. Among these applications, the field of chemical sensors is one of the most critical aspects.

A chemical sensor is composed of two important components, first one is a recognition element and second one is a transduction element (136,137). The recognition element is responsible for selectively sensing and binding the target analyte in an often complex sample. The transducer then converts the chemical signal generated upon analyte binding into an easily quantifiable electrical signal. Depending upon the mode of signal transduction chemical sensors can be classified as mass sensors, thermal sensors, optical sensors and electrochemical sensors (138). In this review article, the main focus is on the electrochemical process that implies the transfer of charge from an electrode to another phase, which can either be a solid or a liquid sample. Both the electrode reactions and/or the charge transport can be modulated chemically and serve as the basis of the sensing process.

Electrochemistry is one of the tools for attaining such information where chemical sensing is realized over a chemically modified electrode (CME) (139). The design of chemically modified electrodes has opened up unique analytical possibilities in this direction. CMEs are prepared by modification of a chemically unpredictive conducting or semiconducting material surface or bulk into chemically predictive one by means of a selected monomolecular, multimolecular or polymeric chemical film, or a composite material. A CME has chemical, electrochemical and/or optical properties that are different from those of unmodified ones and are suited for a particular function. They are inherently sensitive and selective towards the target electroactive species and are fast, accurate, compact, portable and cost effective. Amperometry, Potentiometry and Conductometry are the electroanalytical techiques involving measurement of current, potential and conductance of a cell respectively. Based on these modes of transduction, the electrochemical sensors are usually categorized as amperometric, potentiometric and conductometric sensors.

From the past few years, efforts have been made to utilize nanostructured polymers [such as polyaniline (23,24), polyethyleneimine (25) and poly(indole-6-carboxylic acid) (26)], organically modified silicates (53-55) and nanostructured zinc oxide (92,93,95) either as selective layer or as transducer to fabricate electrochemical sensors. The properties of nanostructured materials such as high surface to volume ratio, ability to be functionalized, having favorable electronic and thermal features and electrocatalytic efficiency attracted considerable attention for their application in assembling electrochemical sensors / biosensors. Here our main focus is on the electrochemical sensors based on different kinds of nanostructured materials and are elaborated in depth vide infra. Different types of electrochemical sensors based on nanostructured material are as follows:

5.1. Amperometric sensors

Amperometric sensors measure the current produced during the oxidation or reduction of a product or reactant usually at a constant applied potential. The important parameter that affects the functioning of amperometric sensors is the electron transfer between the selective layer and the electrode surface. Mainly three kinds of electron transfer reactions take place between the surface of the transducing electrode and analyte molecules. First one is the direct electron transfer process (Figure 7a). In this mechanism, the analyte molecule is directly oxidized or reduced at the surface of the electrode. Here the role of modified electrode is mainly limited towards improving the sensitivity and/or reducing the overpotential for the oxidation/reduction of the target analyte. The second process is catalytic electron transfer (Figure 7b) facilitated by either an enzyme to impart selectivity or via inorganic catalysts such as transition metal complexes or noble metal nanoparticles to impart amplification in the catalytic signal. The third process involves the use of an entity that mediates the electron transfer process between the modified electrode (Figure 7c) and analyte molecule. The analyte molecules in this case are redox proteins such as glucose oxidase etc. whose redox centers are deeply buried within polypeptide structure. The oxidation/reduction process of latter does not proceed at the surface of conventional metal electrodes because the distance between the redox center and the electrode surface exceeds the distance across which electrons are transferred at a measurable rate. Hence, electrical communication

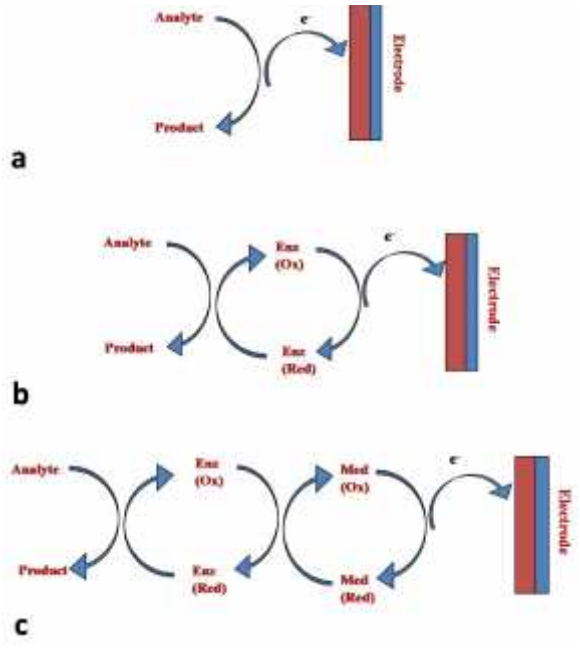

Figure 7. Mechanism of (a) direct oxidation of an analyte over a modified electrode (b) enzyme catalyzed oxidation of an analyte (c) enzyme catalyzed oxidation of an analyte in presence of an electron transfer mediator.

between the redox center of the enzyme and the naked electrode surface requires the presence of electron-transfer relays. Electron transfer mediators such as TCNQ, Fc etc. are electroactive molecules that can undergo oxidation/reduction over the surface of electrode on the application of a suitable potential difference. These molecules penetrate the hydrate enzyme and after approaching its redox center, either transfer or accept the electrons resulting in oxidation/ reduction which is coupled with that of the analyte and in turn, the later gets oxidized or reduced. One of the important properties of a mediator is that although it undergoes several chemical transformations during the reaction, but is regenerated again after the reaction. Electron transfer process may take place in presence of some selective species such as enzymes or other catalysts such as metal nanoparticles etc. The major role of an electron transfer mediator is to lower the overpotential required to carry out the faradic process. Most commonly used mediators are transition metal hexacyanometallates and their derivatives such as Prussian blue etc. Some organometallic electron transfer mediators are also common such as ferrocene and its derivatives. Purely organic electron transfer mediators such as tetrathiafulvalene and tetracyanoquinodimethane are also used to facilitate electron transfer process.

The amperometric sensors are fast, more sensitive, precise and accurate than other electrochemical sensors; therefore these are widely used for the detection of target analytes. Several metallic nanoparticles have been used for the fabrication of amperometric sensors used for the detection of biological analytes (140-142). Catalysis is the most important and widely used chemical application of metal nanoparticles and has been studied extensively. Nanostructured network derived from sol-gel process have been utilized for the immobilization of enzymes and mediators to detect the biological analyte amperometrically (143,144). Other materials like nanostructured polymers (90,91) nanocrystalline metal oxide (145,146) and nancomposites (147,148) are also used for the detection of biological analytes amperometrically.

Pandey *et al* (53-55,149,150,182-186) have been working extensively since long on application of ormosils and carbon paste electrodes for the development of chemical sensors/biosensors for biologically important analytes. Ormosil matrices have been used for the immobilization of glucose oxidase in the development of enzymatic amperometric sensors for glucose detection (130). For this purpose, ferrocene monocarboxylic acid and TCNQ were most prominently utilized as electron transfer mediators. The mediated oxidation of hydrogen peroxide was studied over ormosil electrodes without using any biomolecule employing ferrocene monocarboxylic acid (149), potassium ferricyanide (131) and Prussian blue (125). Ormosil electrodes were also utilized to study the oxidation of dopamine following enzymatic (54) and non-enzymatic (146) reactions. Carbon paste electrodes were also utilized for the preparation of a bienzymatic reactor (150) for studying the oxidation of choline using choline oxidase and acetylthiocholine using acetylcholinesterase. Ding *et al*, Xian *et al* and Ansari *et al* have studied the oxidation of glucose using composites of NiO/AgNF (22), PAni/AuNp (135) and on PVA films (151) respectively. Glucose oxidation has also been monitored using ZnO (152), CeO_2 (153) and CeO_2 (154). Hydrogen peroxide detection employing enzyme has been monitored using *p*-aminobenzene sulfonic acid (155).

5.2. Potentiometric sensors

Potentiometric sensors measure the potential difference between the transducing electrode and reference electrode. The catalytically sensitive membranes are the source of generating potential which is proportional to the logarithm of analyte concentration. Such measurements are performed under zero current flow as the current tends to disturb the equilibrium at the sample membrane interface. Nanostructured network derived from sol-gel process have been utilized for the immobilization of enzymes for the detection of creatinine and urea potentiometrically. Nanocrytalline metal oxide has been used for the development of potentiometric sensor for calcium ion (95).

Pandey *et al* (156) have prepared an electropolymerized film of polyaniline (PAni) on Pt surface and coated it with sol-gel film encapsulating creatininase and urease for potentiometric determination of creatinine and urea respectively. Some of the workers have utilized ZnO films for the determination of calcium ions (95) and glucose (157). Potentiometric determination of glucose has also been monitored potentiometrically employing glucose oxidase using nano-sized SiO_2 (158,159).

5.3. Conductometric sensors

Conductometric sensors measure the change in electrical conductivity or resistance of a film or a bulk material due to the presence of a specific analyte. The

conductometric measurement in an electrolyte is often accompanied by the polarization of electrodes at the operating voltage thus, leading to the occurrence of a faradic or charge transfer process at the electrode surface. While amperometry and potentiometry being the predominantly used electroanalytical techniques, conductometric determination of glucose is reported over the SnO_2, cast over the surface of porous alumina (160).

6. CRITICAL BIOSENSORS BASED ON NANOSTRUCTURED NETWORKS

The unprecedented demand in the research and development of analytical devices for screening, quantification and monitoring of biological species has led to extensive progress in biosensors. Biosensors based on electrochemical transducers combine advantages offered by the selectivity of the biological recognition elements and the sensitivity of electrochemical transduction process. Because of their specificity and catalytic efficiency enzymes and antibodies are highly employed in bioanalytical chemistry. One of the most critical steps in designing a biosensor is immobilization of these biomolecules. A successful matrix should be able to immobilize or integrate these biomolecules stably at a transducer surface and efficiently maintain the functionality of the biomolecules, while providing accessibility towards the target analyte and an intimate contact with the transducer interface. The immobilization strategy ultimately determines the operational stability and long-term use or reusability of a biosensor.

The advent of suitable solid state matrices for immobilizing enzymes and antibodies has reduced many of the limitations associated with the use of soluble forms. Enzymes can be immobilized on transducer or support matrices via physical or chemical means. Physical methods of enzymes immobilization such as adsorption, entrapment, encapsulation, offer the benefit of applicability to many enzymes and may provide relatively small perturbation of native structure and function of the enzyme. Chemical methods of enzyme immobilization include covalent binding and cross linking using multifunctional reagents.

By nature, many biological macromolecules are highly efficient at recognizing specific analytes or catalyzing reactions in aqueous biological media. Liu *et al* (179), Pandey *et al* (180-184), Zucolotto *et al* (186), Manesh *et al* (187) etc. have demonstrated that nanostructured silicate glasses obtained by the sol–gel method and nano-sized conducting polymers can provide such host matrices and that biomolecules immobilized by this method retain their functional characteristics to a large extent. These biocompatible matrices make it possible to retain the specificity and reactivity of biomolecules in the solid state and provide morphological and structural control that is not available when the biomolecules are simply dissolved/ suspended in aqueous media. Furthermore the amorphous nature of the glassy material does not impart a geometric order to the entrapped molecules; many of the characteristics of the liquid state are retained despite the fact that the molecule is trapped in a solid material.

The practice of performing clinical analyses exclusively in the clinical chemistry laboratory has changed a lot. Measurements of analytes in biological fluids are routinely being performed in various locations, including hospital point-of-care environment, by care providers in non-hospital settings and even by patients themselves at home. Biosensors for the measurement of analytes of interest in clinical chemistry are ideally suited for these new applications. They are used for selective determination of various analytes found in blood viz. glucose, urea, lactate, cholesterol etc. for the diagnosis and treatment of a number of diseases. Hence, in this section biosensors based on nanostructured networks are described that take above parameters into consideration. Excellent performances of these biosensors promise to meet the challenges raised by complex clinical samples. Based on different clinical analytes and purposes, clinical biosensors can be categorized as metabolic (glucose, urea, lactate, cholesterol etc.) monitoring biosensors, antibody or antigen (immunosensors), DNA biosensors etc.

6.1. Glucose biosensors

Selective determination of blood glucose is an important health care parameter. The development of analytical tools for efficient and sensitive determination of glucose present in blood becomes particularly critical for patients suffering from diabetes. At present, most of the commercially available glucose sensors are based on amperometric determination of glucose utilizing glucose oxidase for molecular recognition. The main concern when designing enzyme based amperometric biosensors is how to effectively transfer electrons to the electrodes. As mentioned above, Pandey *et al* (53,130,149) have made significant contributions towards scientific research in the area of glucose monitoring employing nanostructured silicates as an immobilization matrix for glucose oxidase and electron transport mediators. Figure 8 represents the generalized scheme for the enzymatic detection of glucose in blood samples using nanostructured materials over electrode surface.

A pictorial representation of above reaction is shown in Figure 9. Figure 10 shows the electrochemical behavior of glucose over ferrocene monocarboxylic acid along with GOx entrapped in ormosil film following redox electrocatalytic mechanism (69). Several reports are also available concerning glucose determination and over various nanostructured matrices viz. polysiloxane (117), PAni coated boron doped diamond (23) and PVA matrix (151) following enzymatic determination of glucose. Reports are also available for non-enzymatic determination of glucose following direct catalytic oxidation using NiO/AgNF (22) nanocomposite and also using mediated oxidation using potassium ferricyanide attached over molecularly imprinted polymer. Some workers have employed gold nanoparticles along with chitosan and carbon nanotubes for determination of glucose (161,162). Application of Pt nanoparticles for the same is also reported (163).

Pandey *et al* have developed and commercialized a system for determination of blood glucose known as PC

Figure 8. Scheme for oxidation of glucose catalyzed by glucose oxidase.

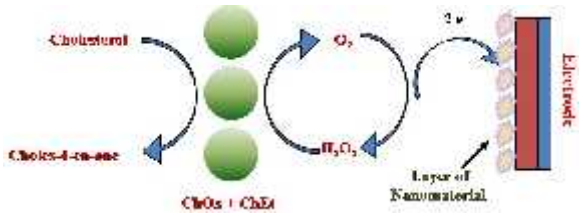

Figure 9. Mechanism of glucose oxidation catalyzed by glucose oxidase (GOx) over an electrode modified with a nanostructured material.

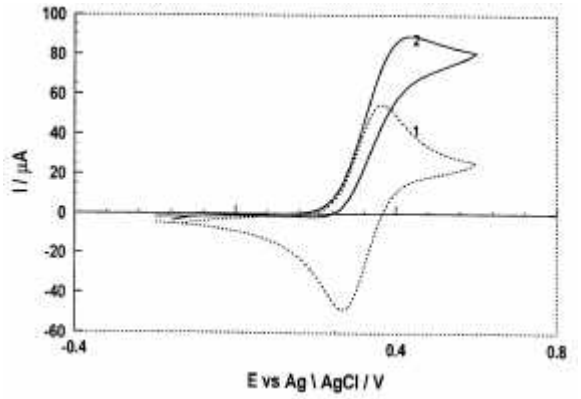

Figure 10. Glucose oxidation over ormosil film encapsulating GOx and ferrocene monocarboxylic acid. Reproduced with permission from (69).

Figure 11. PC Blood Glucometer developed by Sensors Tech.

Blood Glucometer by Sensors Tech., Varanasi that incorporates: (i) LCD based electronic unit with facility of controlling operating potential, for handling various electroactive species incorporated for electron exchange from glucose oxidase through non-mediated, mediated and electrocatalytic modes and time course for attaining target signal display; (ii) disposable test strips are prepared with printing inks among which sensing ink comprises of glucose oxidase and an electron shuttle together with enzyme stabilizer and results in the formation of microband electrodes through screen printing microfabrication technology. Efforts are currently underway to make PC Blood Glucometer (Figure 11) available at cheapest price with better performance of test strips in Indian market. The PC blood glucometer operation is highly user friendly and functions using a preset given on test strip as code number. Self measuring glucose level requires 2 µl blood samples on targeted site where blood sample is automatically applied on sensing area via capillary action. The blood glucose level is displayed in 20 seconds.

6.2. Cholesterol biosensors

Determination of cholesterol has always been considered important because epidemiological and clinical studies associate serum cholesterol with coronary heart diseases (164,165). Due to simple design, specificity and high sensitivity, cholesterol oxidase (ChOx) and cholesterol esterase (ChEt) immobilized electrodes have been found to show potential application as cholesterol biosensor. Figure 12 displays the generalized mechanism for the enzymatic sensing of cholesterol.

The immobilization of ChOx onto an electrode surface is the most critical step for fabrication of a biosensor to lower the limit of detection and obtain high sensitivity and selectivity. In this context, nanostructured materials such as metal nanoparticles, nanostructured polymers and nanomaterials derived from sol-gel process have been used extensively as immobilization matrices (93) for enzymes due to unique properties as described in earlier sections (97,98,53,54,132,135).

Recently, vertically aligned carbon nanotube (CNT) has been used to immobilize enzyme over PVA matrix for the detection of total cholesterol (166). Several reports are available on cholesterol biosensors based on CNT-enzyme-polymer composite formed through electrochemical polymerization (167-172). The reaction for cholesterol determination over electrode surface modified with nanostructured material is represented in Figure 13.

6.3. Urea biosensors

Urea is widely distributed in nature and its analysis is of considerable interest to clinical and agricultural chemistry (173,174). Most of the urea biosensors available in literature are based on detection of NH_4^+ or HCO_3^- sensitive electrodes. The reaction occurring at electrode surface for the determination of urea is shown in Figure 14.

Silicon nano-channels have been used recently with the enzyme urease for biosensor applications to detect and quantify urea concentration (175). Nanocrystalline metal oxide has also been used for the immobilization of urease to detect urea in target samples (92).

6.4. Lactate biosensors

L-lactate is constantly produced from pyruvate by lactate dehydrogenase (LDH) in a process of fermentation during normal metabolism and exercise. L-lactate concentration plays an important role in clinical diagnostics, medicine validation and food analysis. Lactate biosensors are based on enzymes like lactate oxidase and lactate dehydrogenase. The reaction occurring at electrode

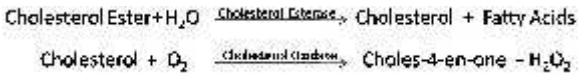

Figure 12. Scheme for oxidation of cholesterol catalyzed by cholesterol esterase and cholesterol oxidase.

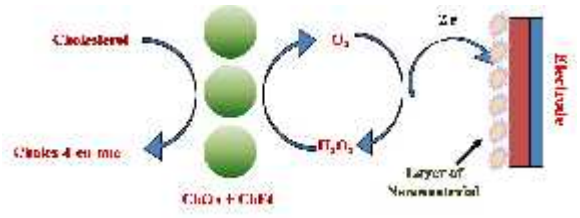

Figure 13. Mechanism of cholesterol oxidation catalyzed by cholesterol oxidase (ChOx) and cholesterol esterase (ChEt) over the surface of a nanostructured matrix.

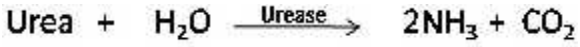

Figure 14. Scheme for reduction of urea catalyzed by urease.

surface for the determination of lactate is shown in Figure 15.

Figure 15. Scheme for oxidation of lactic acid catalyzed by lactate dehydrogenase

Recently lactate biosensor has been developed using composite of conducting polymers and multiwalled CNT for the immobilization of LDH and the results are improved as compared to using conducting polymer alone (176). Recently, study has been performed to evaluate the effect of nano-titania over direct electron transfer form LDH to sol-gel modified with gold and this nano-TiO2-LDH electrode was further employed for the development of lactic acid biosensor (177).

6.5. DNA biosensor

The basis for fabrication of DNA biosensors is hybridization carried out by matching one strand of DNA with that of its complementary to invoke selective determination. These biosensors can be employed for recognition and quantification of targeted DNA in clinical samples. The determination of DNA is widely acknowledged as one of the most recent and exciting application of biosensors and electroanalytical chemistry. This achievement meets the requirements for several critical applications such as sequencing of genome, detection of mutation and identifying genetic diseases. The application of nanostructured networks with desired properties for DNA determination has tremendously improved the sensors technology and opened the gates for determination of a wide range of analytes

Nie *et al* (26) has utilized nano-sized poly (indole-6-carboxylic acid) and performed its modification with single stranded DNA extracted from Hepatitis B virus for amperometric determination of DNA. DNA biosensors are also developed using nanostructued PAni (128) and nano-sized ZnO (145) films using single stranded DNA extracted from *Neisseria gonorrhoeae*.

6.6. Immunosensors

Immunosensors are a class of sensors that combines the electroanalytical chemistry with immunology. These sensors provide information regarding direct electrochemical determination of a wide range of analytes with supreme selectivity and sensitivity. Many pathogenic diseases can be traced by determining the invasive pathogen that acts as antigen. It is well known that the human body produces antibodies (proteins) as an immunological response against such harmful species. The successful analysis these antibodies in various fluids of our body can provide valuable clinical information for diagonising a range of pathogenic diseases including hormonal abnormalities, tumors and metabolic disorders.

Biosensors for antibodies and antigens are being developed with improved sensitivity and selectivity resulting in efficient diagnostic capability. To further match the requirements for clinical practices an effective strategy is to employ nanostructured materials having desired properties in their matrix for superior sensing ability. In this context, Liang *et al* (140) and Shi *et al* (142) have developed amperometric immunosensors based on three dimensional sol-gel network and TiO_2 nanoparticles respectively. Nanocomposites of sol-gel with gold nanowires are also utilized for preparation of an amperometric immunosensor for determination of testosterone (143).

7. CONCLUSIONS AND FUTURE PROSPECTS

In this review, we have highlighted the recent trends in the application of nanostructured network for sensing of biological analytes. These materials are responsible for opening new horizons in the development of electrochemical sensors/ biosensors. Several unique and attractive properties of nanostructured materials present new opportunities for the design of highly sophisticated electroanalytical devices useful for the detection of biological analytes. Due to their high surface area, nontoxicity, excellent biocompatibility and charge-sensitive conductance these materials act as effective transducers in nanoscale biosensing and bioelectronic devices. Electrochemical sensors based on nanostructured materials have several unique features, including high sensitivity, exquisite selectivity, fast response time and rapid recovery (reversibility) and they have great potential for integration of addressable arrays on a massive scale, which sets them apart from other sensors technologies available today.

There is further need for comprehensive efforts to be made in the direction of application of these nanostructured networks for clinical diagnosis. The in depth understanding of these novel materials for electrode modification meeting the requirements of device fabrication is still a great challenge. Furthermore, the

processing, handling and mechanisms controlling the behavior of these nanoscale materials still need to be understood. Analytical tools developed on the basis of these nanostructured networks are expected to have tremendous impact in the area of scientific and technological research.

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Glossary: *Ormosil*: A nanoporous material made through hydrolysis, condensation, polycondensation and densitification of reaction product of organically functionalized alkoxysilane through sol–gel process. *Aerogel*: A very light silicon-based material made by replacing the liquid content of a gel with gas; has an extremely low density and is an excellent insulator. *Xerogel*: A dry gel resulting from the gelation and afterwards ageing of a colloidal suspension (sol). *Conducting polymer*: The polymeric material made through polymerization of organic monomer where the conductivity of the material is the function of dopants. *Carbon paste*: A solid matrix made from the dispersion of fine graphite powder typically to the order of m size in mineral oil. *Electrochemical sensor*: A device designed for the analysis of targeted species based on electrochemical processes i.e., potentiometry, amperometry, conductometry. *Blood glucometer*: An electrochemical biosensor for detecting glucose level in a drop of blood samples. *Non-mediated bioelectrochemistry*: Regeneartion of inherent property of the redox biological molecules through physiological electron acceptor/donor. *Mediated bioelectrochemistry*: Regeneartion of inherent property of the redox biological molecules through nonphysiological electron acceptor/donor i.e., ferrocene, TCNQ, TTF etc. *Chemically modified electrode*: An electrode prepared from a conducting or semiconducting material coated with a selected monomolecular, multimolecular, ionic, or polymeric film of a chemical modifier which by means of faradaic (charge-transfer) reactions or interfacial potential differences (no net charge transfer) exhibits chemical, electrochemical and/or optical properties of the film (IUPAC report, 1998).

Key Words: Nanomaterials, Nanostructured network, Electron transfer mediator, Ormosil, Chemically modified electrode, Sensor, Biosensor, Amperometry, Potentiometry, Conductometry, Review

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