

Chapter 2

Numerous nanomaterials have been explored in the last couple of decades for various technological applications. A single material could not have all the required characteristics for a specific application. Fabrication of composite material systems is an easy way to obtain superior, tailored and valuable properties. Polyaniline (PANI) is one of the most studied conducting polymers due to easy synthesis, inexpensive monomer, environmental stability, highly reversible redox behavior, variable oxidation states, non-toxicity, easy to manipulate morphology, interesting doping chemistry and a wide range of conductivity. Its electronic structure and electrical properties could be easily controlled by the degree of oxidation and protonation. However, it has certain inherent limitation due to less control on reaction, insolubility in common solvents, loss in conductivity at higher pH and poor processability. Nature of acid and associated dopants greatly affects the kinetics of polymerization and morphology. A variety of PANI based materials has been used to fabricate electrochemical sensor systems. Electrochemical detection using enzymatic biosensors represents a reliable and feasible technique due to their simplicity, good sensitivity, high selectivity and low fabrication cost.

In this chapter, we have summarized recent developments in the preparation of different PANI based composite materials, with a special focus on electrochemical sensors/biosensors. We have discussed composite formation along with following class of materials, i.e. polysaccharides, carbonaceous nanomaterials, metal nanoparticles, metal oxides, clays, synthetic polymers and hydrogel.

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2.1 Introduction:

Globally, many research groups are working on the development of novel nanocomposite materials for different applications, including sensor/biosensors. Biosensors are becoming increasingly important as analytical tools because of their simplicity, specificity, fast and unique target recognizing ability, easy operation system, low sample preparation cost, repetitive measurements with miniature and their “on site” usage. A sensor is an analytical device that detects or measures a physical property and records, indicates or otherwise responds to it. A biosensor consists of three main components: the biological receptor/detector, transducer and output device. The active sensing elements selectively interact with the analyte and produce a physical stimulus or biochemical signal which converts into an electrical output using suitable transducer. Amplifier and display system converts the signal into readable form.

Sensors can be classified on the basis of the type of recognizing elements (chemical/biological), mode of transduction (optical, electrochemical, thermometric, piezoelectric) and mode of analyte interaction with the sensing element (affinity sensor and catalytic sensor). Development of a biosensor device requires considering following important issues - chemical receptor/bio-receptor, electrode material, immobilization technique, transducer type, optimization of experimental parameters, real sample/field analysis and prototype design/ technology transfer. Electrochemical sensor response can be measured by voltammetric techniques. Sensors have significance importance in various fields such as clinical diagnosis, environmental monitoring, agro-food analysis, biomedical instrumentation, chemical industries, pharmaceuticals, process and field quality control, harmful gas detection and homeland security (Huang et al. 2004; Attia et al. 2013; Ates et al.

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2013; Maa et al. 2010; Ali et al. 2008; Cosnier et al. 1999; Baker et al. 2017; Kaur et al. 2015; Shahadat et al. 2017; Baker et al. 2017; Srikanth et al. 2016; Jia et al. 2016; Ahmed et al. 2013).

Conducting Polymers are special organic polymers with special physical and electrochemical features e.g. resembling metals, inorganic semiconductors and non-conducting polymers like polyacetylene, polypyrrole, poly-3,4-ethylenedioxythiophene, PANI, polycarbazole, polyindole, polyazepines, polyphenylenevinylene, polythiophene, polyparapenylene. Conducting polymer-based materials have been used for photocatalysts, chemical sensors, super-capacitors, actuators, adsorbents, light emitting diodes, electrochromic devices, anti-corrosion coatings, batteries, electromechanical devices, catalyst support, EMI shielding materials, antibacterial agents, fuel cells, biomedical devices, tissue engineering, dental implants and targeted drug delivery. A literature survey indicates that sensors and conducting polymers are an interesting area of research.

Fabrication of composite material systems is an easy way to tailor synergistic new properties and to compensate the individual deficiencies. The nanocomposite is a material which consists at least two phases with one dispersed in another and one or more of the phases have at least one dimension of the order of 100 nm or less. Nanocomposite displays some unique and new properties, which are not exhibited by either of the pure component phases and even by the conventional composites. Nanocomposites could be classified as polymer nanocomposites, ceramic-metallic nanocomposites, polymer-metallic nanocomposites and ceramic-ceramic nanocomposites. Different instrumental techniques have been used to investigate structural, electrochemical, electrical, morphological properties

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of the composites (Kumar et al. 2017; Gangopadhyay et. al. 2000; Kumar et al. 2010; Song et al. 2013).

2.2 PANI Based Composite Materials

Letheby in 1862 first reported chemical oxidative polymerization of aniline. PANI exists in several different oxidation states, fully reduced leucoemeraldine (C_6H_4NH)_n, protoemeraldine, emeraldine ($[C_6H_4NH]_2[C_6H_4N]_2$)_n, nigraniline and fully oxidized pernigraniline (C_6H_4N)_n. PANI is an extensively studied conducting polymer due to its interesting physicochemical properties like high electrical conductivity, easy synthesis, redox ion-exchange, excellent environmental stability, low cost and simple acid/base doping/dedoping chemistry. Conventionally prepared PANI also exhibited some limitations, such as - poor processing, loss of conductivity at neutral or higher pH, poor reaction control, irregular morphology, hard and brittle, low electrochemical strain, insolubility in most solvents, unsuitable for melting processes, and poor mechanical strength. Different methods have been adopted to overcome the inherent disadvantages and some of them are structural modification of PANI (ring or N-substitution), electrochemical polymerization, colloidal dispersion and blending with materials. Generally, the composite materials were designed in such a way that it could maintain the activity and structural integrity of the enzymes/other biocatalysts and should exhibit fast, stable and sensitive biosensor response. Mass transport to an electrode surface depends on the bulk concentration of analyte, the area of the electrode as well as diffusional and convectional conditions (Thangarathinavelu et al. 1994; Li et al. 2009; Baker et al. 2017; Erden et al. 2017; Tran et al. 2011; Wan et al. 2008). Nanocomposite formation improves inherent properties of the material. Hydrogen bonding

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plays a crucial role in the self-assembling of PANI nanostructure. Nanofibers are found to have superior sensor performance relative to the conventional agglomerated structure due to the large surface area and facilitated charge delocalization. The structural and morphological properties of the nanocomposites are characterized by Fourier-transform infrared, UV-Visible spectroscopy, Raman spectroscopy, X-ray photoelectron spectroscopy and electron microscopy techniques. The electrochemical properties, charge transfer mechanism and sensor performance have been evaluated by voltammetric techniques, amperometric and impedance measurements (Zhang et al. 2004; Rana et al. 2012). PANI based composite materials have been used for various applications (Shahadat et al. 2017; Dallas et al. 2015; Qazi et al. 2014; Yoon et al. 2013; Pillay et al. 2014; Wang et al. 2017; Poppinga et al. 2017; Goor et al. 2017; Wu et al. 2017; Moghaddam et al. 2017; Zhao et al. 2016; Uppalapati et al. 2016; Wang et al. 2004; Wang et al. 2006; Wang et al. 2016; Aydemir et al. 2016).

In this short survey, we have discussed the progress made in the field of PANI based composite materials, especially for electrochemical sensor/biosensors. The study has been systematically categorized on the basis of the type of material used for composite fabrication, such as: polysaccharides, carbonaceous nanomaterials, metal nanoparticles, metal oxides, coordination inorganic complexes and synthetic organic polymers. Important characteristics of some of the PANI based electrochemical sensors are given in the Table 2.1. It includes electrode material, the technique of detection, limit of detection, sensitivity and detection range.

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Table 2.1 Analytical performance of some PANI based sensors/biosensors.

Materials	Method of preparation, Technique of detection	Analytes	Detection Limit (LOD)	Linear Range, Sensitivity	Reference
PANI/Xanthan gum	Grafting, Conductometric	NH ₃	-	-	Pandey et al. 2016
PANI/Agarose/Guar gum	In situ polymerization, Amperometric	NH ₃	-	-	Vaghela et al. 2014
PANI/ Agarose/Guar gum	In situ synthesis, ESI	NH ₃	-	-	Vaghela et al. 2016
PANI/ Carboxymethylpullulan/ CH	Electropolymerization, Potentiometric	Urea	-	0.01 mmol L ⁻¹ , 52 mV/decade	Lakard et al. 2011
PANI/Cyclodextrin	Imprinted sol-gel, Amperometric	l- Phenylalanin e	1.0×10^{-9} mol L ⁻¹	5.0×10^{-7} to 1.0×10^{-4} mol L ⁻¹	Hua et al. 2011
PANI/ β -CD/MWCNT	Ultrasonic agitation, Amperometric	MCPA herbicide /4- chloro-2- methylpheno l	1.1 and 1.9 μ mol L ⁻¹	10 to 50 μ mol L ⁻¹	Rahemi et al. 2015

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PANI/ β -cyclodextrin	Ultrasonic Agitation, Amperometric	Dopamine	1.2×10^{-8} mol L ⁻¹ .	1.0×10^{-7} – 1.0×10^{-3} mol L ⁻¹	Yin et al. 2006
PANI/Calcium Alginate	Electrochemical, Potentiometric	L-arginine	0.038 mM	0.07 to 0.6 mM	Stasyuk et al. 2012
PANI/CMC/Cellulose nanofiber	Electrospinning, CV	Catechol	0.374 μ M	0.497 μ M to 2.27 mM	Fu et al. 2015
PANI/CMC	Electrochemical Polymerization, CV	Cholesterol	1.31mM	0.5–22 Mm, 0.14 mA/mM cm ²	Barik et al. 2010
PANI/Au/CH	Covalent, Amperometric	Cholesterol	37.89 mg dL ⁻¹	50–500 mg dL ⁻¹ /0.86 mA mg dL ⁻¹	Srivastava et al. 2014
PANI/CH	Electrochemical polymerization, EIS	Ochratoxin-A	1 ng/mL	-	Khan et al. 2009
Zinc oxide nanoparticle/CH/MWCN Ts/PANI	Electrodeposition, Amperometric	Xanthine	0.1 mM	0.1–100 mM	Devi et al. 2012
PANI/CNT/CH	Oxidative graft polymerization, Amperometric	Glucose	-	1-20 mM/21 μ A/(mM cm ²)	Wan et al. 2010
Iron oxide nanoparticles/CH-g-PANI	Electrodeposited, Amperometric	Creatinine	1 μ M	1 to 800 μ M/3.9 μ A μ M ⁻¹ cm ⁻²	Yadav et al. 2012
Cu	Covalent immobilization,	Polyphenol	0.156 μ M	1 to 500 μ M	Chawla et al.

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nanoparticles/CH/MWCNTs/PANI/Modified Au electrode	Amperometric				2011
PANI/CH	In situ electropolymerization, Amperometric	Glucose	-	0.06 to 1.83mM	Yavuz et al. 2010
ZnO-NPs/CHIT/c-MWCNT/PANI	Electrochemical, Amperometric	Creatinine	-	-	Yadav et al. 2011
Fe ₃ O ₄ /PANI PANI/CH	Electrodeposition, Amperometric	Catechol	0.4 μM	0.5 to 80 μM	Sadeghi et al. 2015
PANI/MWCNTs/Au nanoparticles	In-situ polymerization, Amperometric	H ₂ O ₂	0.3 mM	3.0 mM to 600.0 mM/3.3 mA/mM	Narang et al. 2011
MWCNTs-COO-/PANI	Layer-by-layer (LbL) technique, SWAS voltammetry	2-chlorophenol	-	-	Silva et al. 2014
PANI/MWCNTCOOH	Chemical oxidation method/CV	H ₂ O ₂	-	86 μM ⁻¹⁰ mM/194.9 μA mM ⁻¹ cm ⁻²	Hua et al. 2011
GOx/Pt-DENs/PANI/CNT	In situ polymerization, Amperometric	Glucose	0.5 μM	1 μM ⁻¹² mM/42.0 μA mM ⁻¹ cm ⁻²	Xu et al. 2009
PANI/MWCNTs	Electrochemical, Amperometric	Choline	0.3 μM	1x10 ⁻⁶ to 2x10 ⁻³ M	Qu F et al. 2005
MWCNTs/PANI	Electrochemical, CV	Daidzein	8 × 10 ⁻⁸	1.0 × 10 ⁻⁷ –9.0 × 10 ⁻⁶	Wang et al. 2016

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			mol L ⁻¹	mol L ⁻¹ / 7.349 μA μmol ⁻¹	
Enzyme/c-MWCNT/PANI/Pt	Electrochemical, Amperometric	Creatinine	0.1 μM	10 to 750 μM/40 μA/mM/cm ²	Yadav et al. 2011
MWCNTs/Cu nanoparticles/PANI	Electrochemical, DPV	Acrylamide	0.2 nM	5 nM to 75 mM /72.51μA/nM/cm ²	Batra et al. 2013
carboxylated MWCNTs/PANI	Electrochemical, Amperometric	Oxalate	3.0μM	8.4–272μM/0.0113μA/μM	Yadav et al. 2011
ChOx/PANI/CNT/Au	In situ chemical polymerization, Amperometric	Cholesterol	30 μM	30-280 μM	Shukla et al. 2015
c-MWCNT/PANI	Electrodeposition, Amperometric	AA	0.9 mM	2–206 mM	Chauhan et al. 2011
PANI/polyacrylic acid/MWCNTs	Electropolymerisation, EIS	AA	2.5x 10 ⁻⁷ M	1.0 μM-1.0 mM, 297.0 μA mM ⁻¹	Tiwari et al. 2012
Fe ₃ O ₄ NPs/cMWCNT/PANI/Au	Electrodeposited, Amperometric	Phenolic compounds	0.03 μM	10–500 μM	Rawal et al. 2012
PANI/GNs	Interfacial polymerization method, CV	H ₂ O ₂ and glucose	50 nm	857 μA mM ⁻¹ cm ⁻²	Qiu et al. 2012
GR/PANI	In situ polymerization, CV	4-aminophenol	6.5 ×10 ⁻⁸ M	604.2 μA mM ⁻¹	Fan et al. 2011

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GrO/PANI/HRP	Electrochemically, CV	Artesunate	0.012 ng mL ⁻¹	0.05–0.40 ngmL ⁻¹ , 0.15 mA ngmL	Radhapyari et al. 2013
PANI/GNO	Chemical polymerization, CV	DNA sensing	-	2.08 × 10 ⁻¹⁶ M	Yang et al. 2014
PANI/Gr	In situ electrochemical, I-V plot	Hydrazine	~15.38 mM	~32.54 × 10 ⁻⁵ A cm ⁻² mM ⁻¹	Ameen et al. 2012
PANI/Gr/Ferrocenecarboxylic acid	Electrochemical deposition, DPV	Nitrite	0.02 mmol L ⁻¹	0.4 mmol L ⁻¹ ca. 300 mmol L ⁻¹	Wei et al. 2015
PANI/Gr	Electrochemical, Amperometric	Glucose	2.769 μM	10.0 μM to 1.48 mM, 22.1 μA mM ⁻¹ cm ⁻²	Feng et al. 2015
GP/PANI	Electrochemical, FI-amperometric system	AA	0.027 mM	0.05–0.50 mM, 4.5754 mA.mM ⁻¹	Norfun et al. 2016
Gr/CuPc/PANI	Electrolytic exfoliation, CV	AA	8.3 μM	100 μM to 3.6 mM, 22.92 μA M ⁻¹ cm ⁻²	Pakapongpan et al. 2013
Gr/Cu phthalocyanine/PANI	Electrolytic exfoliation, CV	AA	6.3 × 10 ⁻⁸ M	5 × 10 ⁻⁷ to 1.2 × 10 ⁻⁵ M, 24.46 μA mM ⁻¹	Pakapongpan et al. 2014
GO/PANI	In situ electrochemical, Amperometric	H ₂ O ₂	18.16 μA mM ⁻¹	0.02 to 8 mM, 257.04 μA mM ⁻¹ cm ⁻²	Du et al. 2017
PANI/Graphitized nanodiamond	In situ interfacial polymerization, I-V plot	Urea	0.05 mg mL ⁻¹	0.1 to 0.9 mg mL ⁻¹ , 381.5 μA (mg mL ⁻¹) ⁻¹ cm ⁻²	Kumar et al. 2017

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Au nanoparticles/AgCl@PANI	In-situ chemical polymerization, DPV	H ₂ O ₂	4 pM	4–34 pM	Yan et al. 2008
PANI/Au	Electrochemical deposition, CV	Anticancer drug gemcitabine	0.031 ngmL ⁻¹	0.10 to 1.10 ngmL ⁻¹	Radhapyari et al. 2015
(AuNPs)/PANI/AgCl/Gelatin	In-situ chemical polymerization, Chronoamperometry	Glucose	-	0.05 to 0.75 mM	Ozdemir et al. 2010
Au nanoparticle/PANI	Drop-casting method, EIS	Glucose	0.1 mM	0.3 to 10 mM	Ahammad et al. 2016
PANI/Au	Surface graft polymerization, DPV	Dopamine	0.1 μM	200 to 0.3 Mm, 3.5 ± 0.4 pA/μM	Zhang et al. 2009
PANI/Au	Electropolymerization, Amperometric	Glucose	5.8 μM	17.6 μM to 1 mM, 13.8 μA·mM ⁻¹ ·cm ⁻²	Tamer et al. 2011
PPyNPs, PANI	Microemulsion method, CV	Glutamate	0.1 nM	0.02 to 400 mM, 533 nA/μM/cm ²	Batra et al. 2014
PANI/poly (allylamine)/PAA	Electropolymerization, Amperometric	Uric Acid	-	-	Wathoni et al. 2014
PANI/Polyacrylic acid	Chemical polymerization, CV	H ₂ O ₂	0.02mM	0.04 to 12 Mm, 417.5 μA/mM-cm ²	Hua et al. 2011

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AuNPs/MWCNT/PANI	Electrodeposited, Amperometric	H ₂ O ₂	3 μ M	3.0 μ M to 600.0 μ M, 3.3 mA μ M ⁻¹	Chauhan et al. 2011
PANI film/Au Nanoparticles	Electropolymerization and electrochemical deposition, CV	DNA biosensor	0.1 nM	0.2–10 nM	Saberi et al. 2013
AuNPs/PANI	Droplet method, Chronoamperometry	D-Glucosamine	1.45 mM	0.5–5 mM, 7.42x10 ⁻³ A mol/L/cm ²	Ngam et al. 2016
Au nanoparticles/PANI/Poly dopamine	Self-polymerization, Amperometric	AA	4.0 x10 ⁻⁷ M	1.0 x10 ⁻⁶ to 1.9x 10x ⁻³ M	Li et al. 2011
Molecularly Imprinted Polymer-Conducting Polymer	Photochemically grafted, CV	Catechol and Dopamine	144 μ M, 228 nM	228 nM to 144 μ M	Lakshmi et al. 2009
PANI/NF	In situ polymerization, Amperometric	DNA detection	-	150 x 10 ⁻¹² to 1 x 10 ⁻⁶ mol L ⁻¹	Spain et al. 2011
AuNPs/PANI	Electrochemically deposited, Amperometric	Glucose	-	50 to 300 mg/dl	Pandey et al. 2008
PANI/Au Nanoparticle	Layer-by-layer method, CV	DNA	-	-	Tian et al. 2004
PANI/Au nanoparticle	Electrochemical polymerization, CV	H ₂ S	0.1 ppb	0.1-100 ppb	Shirsat et al. 2009
PANI/Au	Self-assembly procedure,	AA	5.0 X 10 ⁻⁷	3.0 X10 ⁻⁶ to 2.0 X10 ⁻⁴ M	Xu et al. 2010

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nanocomposites	CV		M		
AuNPs/PANI/AgCl/Gelatin	Electrochemical, Amperometric	Glucose	0.25 mM	0.05 to 0.75 mM	Ozdemir et al. 2010
Au Nanostar/PANI	In situ polymerization, Conductometric	Ammonia Gas	-	-	Kumar et al. 2015
Au Nanoparticles/PANI	Chemical polymerization, Amperometric	Glucose	5.0×10^{-7} M	1.0×10^{-6} to 8.0×10^{-4} mol/L	Xian et al. 2006
Gr/PANI/Au nanoparticles	In situ polymerization, Amperometric	Glucose	0.6 μ M	4.0 μ M to 1.12 mM	Xu et al. 2014
PANI/AuNP	Template free method, Amperometric	Glucose	1 μ M	1 mM to 20 mM, 14.63μ A $\text{mM}^{-1} \text{cm}^{-2}$	Chowdhury et al. 2014
PPy/PANI/Au composite	Chemical method, CV	DNA sensing	1.0×10^{-13} M	1×10^{-6} and 1×10^{-13} M	Wilson et al. 2012
AuNPs/PANI Nanofiber	Interfacial polymerization, Amperometric	H ₂ O ₂	3 μ M	8.34 mAM^{-1}	Hung et al. 2010
AgCl@PANI	One step synthesis, CV	Dopamine, AA	-	-	Yan et al. 2010
PANI nanowires/Ag nanoparticles	Electrochemically, Conductometric	H ₂ O ₂	5 mM	0.0126 μ A $\text{mM}^{-1} \text{s}^{-1}$	Song et al. 2014
Ag nanoparticles /PANI	In situ polymerization, Relative humidity	Humidity sensing	-	-	Fuke et al. 2010

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Ag nanoparticles/PANI	Electrochemical, Amperometric	Urea	-	-	Crespilho et al. 2009
Laccase/AgNPs/cMWCNT/PANI/Au electrode	Electrodeposited, Amperometric	Polyphenol	0.1 μ M	0.1–500 μ M	Rawal et al. 2011
Ag nanoparticles/PANI	Layer-by-Layer (LbL) deposition, UV	Ammonia	0.2038 mM	0–800 mM	Detsri et al. 2015
Ag nanoparticles/decorated PANI/Tungstophosphate	Sol-gel mixing, I-V method	Hydrazine	2.8 nM	0.01–10000 μ M, 12.5 μ A μ M ⁻¹ cm ⁻²	Rahman et al. 2016
Ag/PANI	In situ reduction, DPV	Hydrazine	-	0.0010–0.50 mM	Singh et al. 2011
Ag decorated PANI/Zeolite	Self-assembly, CV	Lindane	5 nM	10 nM to 900 μ M, 1.24 μ A mM ⁻¹ cm ⁻²	Kaur et al. 2015
PANI/Ag	In situ polymerization, LSPR Probe	AA	7.383 \times 10 ⁻¹¹ M	10 ⁻⁸ M to 10 ⁻⁶ M, 45.1 nm log ⁽⁻¹⁾ M	Shrivastav et al. 2016
Cu-NPs/PANI/GCE	Electropolymerization, Amperometric	AA	0.002 mM	0.005 to 3.5mM	Xi et al. 2010
CuNPs)/PANI/Gr	In-situ reduction, Amperometric	Glucose	0.27 μ M	1.0 μ M to 0.96 mM, ~150 μ A cm ⁻² M ⁻¹	Zheng et al. 2016
PANI/Cu Nano Composite	In situ polymerization, CV	Dopamine	-	-	Sathiyar et al. 2017
Graphene/Cu phthalocyanine/PANI	Electrolytic exfoliation, Amperometry.	AA	6.3 \times 10 ⁻⁸ M	5 \times 10 ⁻⁷ to 1.2 \times 10 ⁻⁵ M, 24.46 μ A mM ⁻¹	Pakapongpan et al. 2014

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PANI/Cu nanocomposite	Electropolymerization, Amperometric	H ₂ O ₂	0.33 μM	1.0 to 500 μM	Liang et al. 2017
Ammonium ion-selective Cu/PANI nanocomposite	Electrochemical, Amperometric	Creatinine and Urea	0.5 μM	1-125 μM/85 ± 3.4 mAM ⁽⁻¹⁾ cm ⁽⁻²⁾ , 112 ± 3.36 mAM ⁽⁻¹⁾ cm ⁽⁻²⁾	Zhybak et al. 2016
rGO/PANI/PtNP/GCE	Electrochemical, Amperometric	H ₂ O ₂	1.1 μM	0.02-8 mM , 257.04 (μA mM ⁻¹ cm ⁻²)	Du et al. 2017
Pt/PANI	Directly synthesized in films, Amperometric	Glucose	0.10 μM	5.9 μM to 0.51 mM, 5.5 μAmM ⁻¹	Song et al. 2010
(PtNPs) on PANI/Mesoporous silica film	Electrodeposition, Amperometric	H ₂ O ₂	0.24 μM	1.0 μM to 2.0 mM, 50 μA mM ⁻¹	Ding et al. 2015
Pt Nanoparticle/PANI	Mixing, Amperometric	Glucose	0.7 μM	0.01 to 8 mM, 96.1 μA ³ mM ⁻¹ cm ⁻²	Zhai et al. 2013
PANI/Au or Pt	Solid-state method, Amperometric	H ₂ O ₂	-	-	Jamal et al. 2013
Pt/PANI/GNs	Interfacial polymerization, CV	H ₂ O ₂ , Glucose	50 nm	857 μAmM ⁻¹ cm ⁻²	Qiu et al. 2012
Poly(ANI-co-Py)/Pd	Electrochemical, CV	Hydrazine	0.38 μM	3 mM and 0.01 M, 98.31 μAmM ⁻¹	Ali et al. 2017

2.3 PANI and Polysaccharides

Polysaccharides are abundantly available renewable polymers on the earth. They are used for energy storage or as structural polymers of microbes, animals and plants. Polysaccharides have sugar molecules bonded with a glycosidic linkage and other functional groups. They have following characteristics: low density, biocompatibility, wide range of molecular weights and solubility, poly-dispersity, linear or branched structures, mono/polyfunctional, high level of chirality, water soluble/insoluble, low toxicity, environment-friendly and also are abundantly available. Polysaccharide-based materials are green substituent of synthetic polymers and they have been used for various technological applications including biomedicine, energy production, storage, electrical devices, separation, membrane technology, water treatment, drug delivery, sensors, batteries, electrospinning, magnetic shielding material and pharmaceuticals.

Blends of PANI have been prepared along with different polysaccharides e.g. starch, carboxymethyl cellulose (CMC), cellulose acetate (CA), chitosan (CH), guar gum, carrageenan, alginate, xanthan gum and others. These materials are biodegradable, conductive, electro-active and environment-friendly (Dumitriu et al. 2004; Yingying et al. 2015). Starch is white, granular, soft and tasteless organic chemical, comprising of glucose monomers joined in α 1,4 linkages having chemical formula $(C_6H_{10}O_5)_n$. Starch-based hybrid materials have been extensively used both in biological and non-biological applications. We have studied the morphological and spectroscopic properties of different composite systems involving starch, CMC, CA and CH (Gautam et al. 2016). We have developed a ternary nanocomposite material using PANI, MWCNTs and Starch for sensor application (Gautam et al. 2017). Zhang and coworkers reported PANI/starch for capacitance application (Zhang et al. 2013).

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Dextran is a hydrophilic biopolymer of glucopyranosyl subunits. Hydroxyl groups could easily modify carboxyl groups and other functional groups. MWCNTs/ β -cyclodextrin/PANI modified glassy-carbon electrode has been used for dopamine (DA) sensing in the presence of AA. Formation of a supramolecular complex between β -CD and DA promotes sensitive detection of DA (Yin et al. 2006). Chlorophenoxy herbicide "MCPA" was electrochemically determined in natural waters using multi-walled carbon nanotubes (MWCNTs) with incorporated β -cyclodextrin/PANI modified glassy carbon electrode (Rahemi et al. 2012). Hasegawa and coworkers reported nuclear magnetic resonance and optical absorption spectroscopic studies on PANI/ β -cyclodextrin complex and they conclude that the inclusion weakens the intermolecular π - π interaction and enhances the accompanying twisting motion, consequently leading to a blue-shift of UV-vis absorption (Hasegawa et al. 2012). L-phenylalanine was determined in blood sample by using PANI/ β -cyclodextrin/MWCNTs imprinted sol-gel films (Hu et al. 2011). PANI/ β -cyclodextrin/MWCNTs modified glassy-carbon electrode has been used for determination of dopamine in the presence of AA (Yin et al. 2006). "Hairy urchin"-shaped PANI was prepared by microemulsion polymerization technique in the presence of β -cyclodextrin. The determination of l-phenylalanine in a blood plasma samples has been proposed by PANI/ β -cyclodextrin/MWCNT imprinted sol-gel film. The response mechanism of the imprinted sensor for l-phenylalanine was based on the inclusion interaction of β -cyclodextrin and molecular recognition capacity of the imprinted film for l-phenylalanine (Hua et al. 2011).

Pandey et al. reported microwave assisted synthesis of PANI/Xanthum gum for ammonium detection (Pandey et al. 2016). PANI/agarose/Guar gum film was formed by in situ polymerization of aniline using potassium dichromate as an oxidant. Agar rose-guar gum gives mechanical support to PANI and acts as a microporous template (Vaghela et al. 2014). L-

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arginine amperometric biosensor based on recombinant human arginase-I and urease was proposed (Stasyuk et al. 2012).

Carboxymethyl cellulose (CMC), one of the most important cellulose derivatives, is crucial for many scientific investigations and industrial applications. CMC is an attractive semi-natural polymer. It is nontoxic, soluble, chemically resistant, and physiologically inert. It also has anionic character, binding properties, biocompatibility and membrane forming ability. Hydroxyl groups and bonded water molecules provide a compatible micro-environment to immobilized biocatalysts. PANI nanorods/CMC modified has been used for catechol detection. The composite material has large electroactive surface area, facile electron and mass transport (Fu et al. 2015). Cholesterol was detected by immobilizing Cholesterol oxidase onto PANI/Carboxymethyl cellulose (PANI-CMC) deposited onto indium-tin-oxide (ITO) coated glass plate using glutaraldehyde and as a cross-linker. The proposed sensor exhibits no interference response of glucose, uric acid, ascorbic acid (AA) and urea (Barik et al. 2010).

CH has good film-forming properties, high mechanical strength, adhesion and its gel easily cross-linked with glutaraldehyde, exhibited good swelling properties but poor electrical conductivity. It can be used for a wide range of applications such as controlled delivery systems, packaging wastewater treatments, separation membranes and biosensors. The composite of conducting polymers and CH are conducting, biodegradable and biocompatible (Khairkar et al. 2014). CH/PANI/Au nanocomposite has been used for cholesterol detection, using cholesterol oxidase as biocatalyst (Srivastava et al. 2014). PANI/CH hybrid conducting biopolymer film was used to immobilize rabbit antibody (IgGs) to detect Ochratoxin-A with linear increase in impedance upto 10 ng/mL. Relative change in *RCT* was higher in PANI-CS due to carboxylic and hydroxyl functionalities (Khan et al. 2009). Zinc oxide nanoparticle/CH/MWCNTs/PANI

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composite and xanthine oxidase modified platinum electrode was used to detect xanthine (Devi et al. 2012). A trienzyme biosensor system has been fabricated using creatinine amidohydrolase, creatine amidohydrolase and sarcosine oxidase immobilized on ZnO-NPs/CHIT/c-MWCNT/PANI/Pt electrode to detect creatinine in human blood serum (Yadav et al. 2011). Au nanoparticles/CH/MWCNTs/PANI-modified Au electrode was used for amperometric sulfite biosensor. Sulfite oxidase (SOx) was extracted from leaves of *Syzygium cumini* (Jamun) and immobilized covalently onto the composite material. The sulfite biosensor can be used to detect sulphite in fruit juices and alcoholic beverages (Rawal et al. 2011). PANI/CH/CNTs based amperometric sensor reported for glucose using glucose oxidase (Wan et al. 2010). Iron oxide nanoparticles/CH/Graft-PANI composite material was used to immobilize creatininase, creatinase and sarcosine oxidase to detect creatinine in the serum (Yadav et al. 2012). PANI/CH composites by in-situ electropolymerization at ITO electrode and applied for glucose sensing. The granular porous surface of the composite film was suitable for the immobilization of biomolecules (Yavuz et al. 2010). A potentiometric biosensor was developed by physisorbed urease onto a layer-by-layer assembled PANI carboxymethylpullulan and CH films (Lakard et al. 2011).

2.4 PANI and Carbonaceous Nanomaterials:

A variety of nanoparticles have been used as nanofiller in the polymer matrix to develop hybrid nanocomposite materials. Carbonaceous nanomaterials exist in a wide variety of forms including, carbon, graphite, diamond, highly oriented pyrolytic graphite (HOPG), graphene, carbon nanotubes, carbon nanofibers and graphene oxide. They exhibit extraordinary physical-chemical properties due to their high surface area, lightweight, low-cost, versatility, good

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electrical conductivity, controlled pore size, uniform distribution, compatibility with other materials and chemical stability at wide temperature range.

CNTs are cylindrical carbon nanostructures, having good mechanical strength, electrical conductivity, properties, large specific area, rich hollows and layered structure. On the basis of the tube numbers, CNTs are called as SWNTs, DWNTs, and MWCNTs. A large number of papers described the properties and applications of CNT based materials (Akbar et al. 2009; Ozoemena et al. 2009; Khan et al. 2017; Suvarnapa et al. 2017; Forsyth et al. 2017; Mao et al. 2017; Zhao et al. 2017; Huang et al. 2012).

Carbon nanomaterials facilitate the rapid charge transfer. They act as conducting nanofiller, nanowire and as nanotemplate. Polymerization in the presence of carbon nanotubes leads to the interaction between the quinoid ring of PANI and the MWCNT, thus a more planar conformation of chains and a tubular core like structure is observed. PANI/CNTs exhibited large surface area, more solubility, improved mechanical and electronic properties and high thermal stability (Miyagawa et al. 2005; Oueiny et al. 2014; Srikant et al. 2016).

Carbonaceous nanomaterials have been used for waste water treatment, adsorption, photocatalysis, disinfection, membranes process monitoring and desalination (Shan et al. 2017). Carbon nanotubes could be easily dispersed in aqueous solution and used for thin and uniform layer on screen printed transducer. Functionalized and polymer coated CNTs have been used to immobilize enzymes, antibodies and DNA for biosensor fabrication. CNT coated screen printed electrode was used for breast cancer biomarker analysis (Nawaz et al. 2016). PANI/MWCNT electrode was used for peroxide sensing (Chauhan et al. 2011; Hua et al. 2011). Good conductivity and better electrochemical signal and strong enzyme affinity synergistically promoted the electron transfer between the active site of the enzyme and the electrode surface.

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Layer-by-layer assembly of functionalized carbon nanotube and PANI were used for the 2-chlorophenol (Silva et al. 2014) and choline (Qu et al. 2005). Nanotubes were functionalized by carboxylic groups by treating with concentrated sulfuric and nitric acid. (c-MWCNT/PANI) layer electrochemically modified Au electrode was used to immobilize Ascorbate oxidase (obtained from *Lagenaria siceraria* fruit) for AA sensing (Chauhan et al. 2011). An AA sensor based on PANI/Polyacrylic acid/Nafion/MWCNTs modified platinum electrode has also been reported (Tiwari et al. 2011). It was found that well aligned MWCNTs/PANI Langmuir–Blodgett film show better sensor performance than randomly aligned material (Wang et al. 2016). GOx/Pt-DENs/PANI/CNT electrode exhibits excellent response performance to glucose (Xu et al. 2009).

Creatinine biosensor was fabricated using enzyme/c-MWCNT/PANI modified Pt electrode in human serum (Yadav et al. 2011). PANI/MWCNTs nanocomposite was used to fabricate oxalate biosensor and it was successfully applied to detect oxalate in human urine sample (Yadav et al. 2011). MWCNT-COOH/Cu nanoparticle/PANI nanocomposite was electrodeposited onto pencil graphite electrode for acrylamide sensing using covalently immobilized Hemoglobin (Batra et al. 2013). PANI/CNT composite electrode was used to detect cholesterol using cholesterol oxidase (Shukla et al. 2015). Iron oxide nanoparticles decorated carboxylated MWCNTs/PANI composite electrodeposited onto an Au electrode was used for the construction of an amperometric biosensor for phenolic compounds (Rawal et al. 2012).

Graphene is two-dimensional (2D) monolayer carbonaceous nanomaterial having sp^2 hybridized carbon atoms. Its high surface area and high in-plane electrical conductivity make it a promising material for flexible electronics, energy storage and sensor applications. Intrinsic corrugations, defects, vacancies influence its reactivity (Celina et al. 2017). Graphene can be

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prepared by mechanical exfoliation method, exfoliation of graphite in organic solvents, epitaxial growth, chemical vapor deposition, the exfoliation and reduction of chemically oxidized graphite and mechanical exfoliation. Graphene oxides have abundant oxygenated functional groups on their basal plane and edges which make it highly water dispersible. The fabrication processes of GO breaks the π -conjugated network of PANI. Hence, PANI/Graphene exhibited good water-dispersing ability (Wang et al. 2016). PANI/GNs (Graphene Nano composites) was prepared by interfacial polymerization method and used for electrocatalytic oxidation of methanol. H_2O_2 and glucose were used as two model analytes to demonstrate the sensing performance of the material (Qiu et al. 2012). PANI/Graphene nanocomposite was used for 4-aminophenol and paracetamol (Fan et al. 2011), artesunate (Radhapyari et al. 2013), nitrite (Wei et al. 2015), glucose (Feng et al. 2015), AA (Norfun et al. 2016), tumor biomarkers (Ren et al. 2017), urea (Kumar et al. 2017).

2.5 PANI and Metal Nanoparticles:

Metal nanoparticles (NPs) exhibited significantly different properties than bulk metal due to their high surface-to-volume ratio. NPs is a fascinating area of research and they have potential use in biotechnology and biomedical applications, magnetic separation, sensors, drug delivery, optical device, diagnostic imaging etc. (Corbierre et al. 2005; Moghaddam et al. 2014). Metal ions precursors convert to zero valent metal atoms in reducing environment, which finally gives rise to metal NPs. Metal NPs are synthesized by various methods such as- chemical reduction, optical reduction, hydrogel method, sedimentation method, UV and gamma irradiation and biosynthesis method. Metal nanoparticles are formed as vander Waals nanoclusters, ionic nanocluster and metal nanoclusters. In the absence of any counteractive repulsive forces, the vander Waals forces between the two metal NPs lead to coagulation. Size and shape of NP depend on the method of

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preparation. The photo-reduction process of metal is an easy, environment-friendly and contamination-free method for preparation of metal NPs having desired shape and size with uniform distribution within the matrix (specific particle size depends on the time of irradiation). Metal NPs could be stabilized by using surfactant, capping agent, polymers, suitable functional groups, solid supports and ligands. Functionalized nanoparticles could easily communicate with biomolecules.

NPs act as conductive junctions between the PANI chains and thus lead to better electrical conductivity of the composite. The electrical conductivity of such composites might also depend upon the molecular structure and crystallite size of the conductive polymer matrix. NPs have been used to decorate the surface of polymer and they also get uniformly dispersed deep inside the polymer fibers. PANI/Au nanorod particles film was used as an excellent matrix due to the large specific surface area and better electroactivity. The chemical interaction between Au and PANI is depended upon the size of NPs. PANI and Au NPs act as donor and acceptor and the interaction indicated the formation of the strong bond between positively charged nitrogen and slightly negatively charged Au NPs. It was used for the amperometric determination of glucose using glutaraldehyde to immobilize glucose oxidase (Tamer et al. 2011). PANI nanofibers could be decorated by metal NPs through in situ reduction of selected metal salt. The resulting PANI/metal NP composites comes out to be a promising material for ultrafast nonvolatile memory devices and for chemical catalysis. Au NPs enhance the electrical and electrochemical properties of PANI at higher pH. AuNPs/AgCl-PANI composite material was used for glucose biosensor. The composite exhibited high enzyme loading capacity and improved catalytic oxidation and reduction of H_2O_2 in a linear range of the order of pico molar (Yan et al. 2008).

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Au NPs/AgCl/PANI/gelatin nanocomposite was used to immobilize pyranoseoxidase to detect glucose (Ozdemir et al. 2010). Au NPs/PANI composite film was used for non-enzymatic impedimetric glucose sensor. The DC potential was applied in the EIS measurements and amperometric measurements. The inherent problems due to high DC overvoltage applied in amperometric measurements can be overcome in impedimetric method. EIS method was found to be better than amperometric method (Ahammad et al. 2016). Au NPs were self-assembled due to electrostatic interaction between amine groups of PANI at ITO electrode. Amino groups covalently bonded glucose oxidase in the near vicinity of NP, having facilitated the charge transfer during glucose sensing (Pandey et al. 2008).

PANI/Au NPs were used for electrochemical detection of Gemcitabine (an anticancer drug) (Radhapyari et al. 2015). PANI/Au nanocomposite modified nanoelectrode was used for dopamine nanosensor. Differential pulse voltammogram indicates that dopamine could be selectively determined in the presence of AA (Zhang et al. 2009). PANI/MWCNTs/Au NPs modified Au electrode was used for non-enzymatic H₂O₂ sensor (Narang et al. 2011). Based on a dual signal amplified strategy PANI/Au NPs film was used to fabricate electrochemical DNA biosensor (Saber et al. 2013). PANI nanofibres over Au electrode were decorated with Au NPs (grown chemically). Au NP increases the total area available for DNA immobilization on the Au electrode. The immobilized single-stranded DNA was allowed to hybridize with a complementary target strand (uniquely associated with the pathogen *Staphylococcus aureus* that causes mastitis) (Spain et al. 2011).

Au NPs/PANI/Polydopamine hybrid composite was synthesized by a two-step reaction, self-polymerization of dopamine and the formation of AuNPs, where both pDA and aniline act as reducing agent (Li et al. 2011). Au NPs and PANI modified carbon paste electrode was used

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for D-glucosamine (Ngam et al. 2016). PANI/Au NPs was used to detect hydrogen sulphide, excellent limit of detection 0.1 ppb wide dynamic range 0.1–100 ppb (Shirsat et al. 2009).

Layer-by-layer deposited films of PANI and mercaptosuccinic-acid-capped Au NPs film was used for electrocatalytic oxidation of NADH and DNA hybridization (Tian et al. 2004). Au NPs improves the electrocatalytic oxidation of AA (Xu et al. 2010). The reactivity and sensitivity was found to be significantly dependent on the shape and size of Au NPs. Au nanostar/PANI composite material exhibited ultrasensitive detection of ammonia. Au nanostars (contains Cu, have rough surfaces and large numbers of active sites due to tips, sides, corners and large surface area-to-volume ratios due to their branched morphology (Kumar et al. 2015). Sulfonated Au NPs/PANI/GOx/Nafion was used to fabricate a novel glucose biosensor and the large surface area of composite system exhibited fast diffusion of analytes and electrolytes (Xian et al. 2006). Graphene/PANI/Au NPs modified glassy carbon electrode based biosensor offered favorable microenvironment to facilitate direct electron transfer between glucose oxidase and electrode (Xua et al. 2014). Covalent attachment of biomolecules on functionalized Au-PANI matrix could be used as a general biosensing platform to detect glucose, using chronoamperometric and flow cell methods (Chowdhury et al. 2014). A Polypyrrole/PANI/Au nanocomposite film was used for electrochemical DNA sensing. The microdispersed Au NPs in the polymers nanotubes provide good platform for anchoring the thiolated ssDNA without using any complex immobilization chemistry, and it was used for the fabrication of polymer based DNA sensors (Wilson et al. 2012). Ultra-fine and uniform Au NPs were selectively reduced on water-dispersible PANI nanofibers without using protective agents. The strong interaction between AuNPs and PANFs enhanced electrocatalytic oxidation of H_2O_2 (Hung et al. 2010). Solid-state method was applied

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for synthesizing PANI/Au, which displayed a fast amperometric response to H_2O_2 and a wide linear range (Jamal et al. 2013).

Ag NPs exhibited electrocatalytic reduction of H_2O_2 and generated hydroxide ions and water, which influenced the conductivity of PANI. Ag nanoparticle/PANI nanowires composite system was used to detect H_2O_2 . The rate of the catalytic reaction was found to be proportional to the number of Ag NPs attached on the surfaces of PANI (Song et al. 2014). Ag/PANI nanocomposite was used to fabricate optical humidity sensor. Smaller particle size leads to greater sensitivity and fast response (Fuke et al. 2010). PANI/Ag nanocomposites have been used as a support matrix for the covalently immobilized α -amylase to hydrolyze starch (Khan et al. 2013). PANI/Ag NPs electroactive membrane was used as an efficient enzymatic bioelectrochemical urea sensor. Ag nanoparticle was stabilized in polyvinyl alcohol and coated over ITO electrode along with urease by the drop method. At higher concentration of NPs, they agglomerated with each other in the polymer matrix. ITO/PANI/PVA/AgNP/urease electrode was used for amperometric measurements of urea where the immobilized enzyme promoted the catalytic conversion of urea into ammonium and bicarbonate ions (Crespilho et al. 2009). AgNPs/MWCNTs/PANI/Au electrode was used to fabricate Polyphenol biosensor using laccase. It was applied to determine total phenolic content in tea, alcoholic beverages and pharmaceutical formulations (Rawal et al. 2011).

Layer-by-layer deposition technique was used for thin film formation by alternate deposition of Ag NPs, PANI and applied for ammonia sensing (based on color change) (Detsri et al. 2015). Ag/PANI nanocomposite was prepared by in situ reduction of Ag in aniline by mild photolysis at short wavelength (365 nm) radiation from UV lamp for 12 h. X-ray diffraction (XRD) pattern suggests size consistent cubic Ag NPs. The modified electrode was applied for

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electrocatalytic oxidation of hydrazine (Singh et al. 2011). Ag NP/PANI-nanocrystalline/zeolite was developed for the nanomolar detection of lindane in an aqueous solution of methanol with high electro-catalytic activity, stability, sensitivity and selectivity. The sensor exhibited a wide linear range from 10 nM to 900 mM with a limit of detection of 5 nM under the optimum conditions. The analytical performance of the developed sensor was demonstrated for the determination of lindane in different water bodies with satisfactory recovery (Kaur et al. 2015).

Cu NPs/PANI composite was prepared by in-situ electrochemically deposited on a novel ionic liquid medium. Cu-NPs were uniformly dispersed and firmly attached to the PANI surface. Cu-NPs/PANI/GCE was used for electrocatalytic oxidation of AA (Xi et al. 2010). Cu NPs/PANI/graphene nanocomposite was fabricated via simple in-situ reduction of Cu precursor in PANI nanofibers under mild conditions followed by mechanical mixing with suspension of graphene (0.5%, 1%, and 2%). The developed composite material was used for the highly selective non-enzymatic glucose sensor (Zheng et al. 2016). CuNPs/CH/carboxylated multiwalled carbon nanotube/PANI-modified (Au) electrode was used amperometric polyphenol biosensor, using immobilized *Ganoderma* sp. Laccase (Chawla et al. 2011). PANI-Cu composite material was used for the excellent electro catalytic sensing of Dopamine (DA) (Sathiyar et al. 2017), H_2O_2 (Liang et al. 2017). Creatinine and urea was detected using Cu/PANI nanocomposite modified ammonium ion-selective electrode. In this work, a combination of creatinine deaminase and urease has been chosen as a model system to determine urea and creatinine. Glycerol and lactitol were used as stabilizers during immobilization of enzymes (Zhybak et al. 2016).

Pt-NPs/rGO/PANI nanocomposite (water soluble) modified glassy carbon electrode, used for non-enzymatic reduction of H_2O_2 , exhibited an expanded linear range, higher sensitivity and

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lower detection limit (Du et al. 2017). An amperometric biosensor based on Pt-NPs/PANI/boron-doped diamond electrode exhibited an excellent response to glucose (Song et al. 2010). Platinum (Pt) NPs decorated PANI/mesoporous silica film exhibited high electrocatalytic activity toward the oxidation of H_2O_2 . Meso-porous silica improves the mechanical strength of PANI. Pt-NPs increase the active surface area of the electrode and facilitated the charge transfer along the PANI granules (Ding et al. 2015). Pt-NPs /PANI hydrogel was used for glucose sensing (Zhai et al. 2013).

Poly(ANI-co-Py)/Pd-NPs composite was prepared by electrochemical polymerization, using mixed monomer solution in 0.5 M $LiClO_4$ /acetonitrile for electrocatalytic oxidation of hydrazine (Ali et al. 2017). Pd-NPs/PANI/SBA-15 was synthesized using an in-situ chemical polymerization method, in which Pd-NPs were homogeneously dispersed and anchored on PANI supported by SBA-15. Pd NPs dominantly immobilized on nitrogen sites in the PANI chains. It was used for electrocatalytic reduction of bromate in an acidic medium (Sun et al. 2015).

2.6 PANI and Metal Oxide/Metal Salt:

Many transition metal oxides/sulfides are attractive semiconducting materials due to low cost, nontoxicity, rich resources and ease of synthesis in various nano-sized dimensions. They exhibited high specific capacitance, good chemical stability, very wide range of electrical resistivity and catalytic or photocatalytic properties. Some commonly used metal oxides used for composite material formation are TiO_2 , ZnO , Al_2O_3 , CuO , Fe_2O_3 , In_2O_3 , SnO_2 , As_2O_3 , MoO_3 and NiO . PANI/Metal composite exhibited semi-crystalline behavior, homogeneous dispersion and reduction in grain boundary volume and the properties are known to be strongly dependent on size, anisotropy and inter-particle interactions. These metal oxides have narrow band gap and

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strong binding affinity with the nitrogen lone pair of PANI. The presence of metallic crystals of metal oxide has strong influence on electrical parameters, band gap energy, catalytic oxidation-reduction process, lowering the charge transfer resistance and enhanced thermal stability (Sharma et al. 2008; Mirmohseni et al. 2012; Alvi et al. 2010; Ozerol et al. 2016; Farias et al. 2000; Majumder et al. 2017). PANI/Iron oxide magnetic nanocomposite was synthesized and characterized using various spectroscopic, structural and electrochemical techniques and was used for catechol detection in tap water samples (Chandra et al. 2013). $\text{Fe}_3\text{O}_4\text{NPs}/\text{cMWCNT}/\text{PANI}/\text{Au}$ electrode was used for determination of phenol content in tea leaves extract (Rawal et al. 2012). The ternary composite material “PANI/ $\text{Fe}_2\text{O}_3/\text{rGO}$ ” was used for the sensitive hydroquinone determination (Radhakrishnan et al. 2015).

PANI/ TiO_2 nanocomposite was prepared by electrochemical polymerization of aniline with Titania added into the solution. The film was used to detect ammonia. Titania increased the sensitivity of the composite and there was 500% increase in resistance on 100 ppm of ammonia (Kunzo et al. 2013).

A PANI/ SnO_2 hybrid material was fabricated using in situ polymerization method and used to detect NH_3 , trimethylamine and SO_2 . Coral-like mesoporous SnO_2 was synthesized by controlling the hydrolysis of SnCl_4 and was used subsequently for removal of the templates by calcination in air (Xiang et al. 2015). $\text{Ag}/\text{SnO}_2/\text{PANI}$ composite was prepared by facile in-situ polymerization and dip technique. Ag doped tin oxide nanofibers were fabricated by electrospinning and subsequently calcinations technique. The developed material was used to determine hydrogen gas. SnO_2 -intercalated PANI nanocomposite was used to detect ammonia gas (Deshpande et al. 2009).

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PANI/MnO₂ nanocomposites modified stainless steel electrode was applied for urea biosensing (Mahajan et al. 2015). PANI/MnO₂/GCE was used for nonenzymatic determination of H₂O₂, with negligible interference from AA, uric acid, l-cysteine and glucose (Lee et al. 2015). NiCo₂O₄/PANI composite and NiCo₂O₄ NPs modified glassy carbon electrodes were used to determine glucose. The nanocomposite was fabricated via a facile hydrothermal treatment followed by a post-PANI coating process (Yu et al. 2016). PANI-coated titanium dioxide-silicon dioxide and CuO-TiO₂-SiO₂ hetero-structure was used to determine ammonia. Excellent sensing properties may be due to the P-N, P-P heterojunctions and a structure similar to field-effect transistors formed on the interfaces between PANI, TiO₂ and CuO (Pang et al. 2017). PANI/Zirconia was used to detect methyl parathion (Wang et al. 2015).

PANI/In₂O₃ have been synthesized using in-situ polymerization method using different concentrations of nano In₂O₃ powder and the composite material was used for determining liquefied petroleum gas. In₂O₃-NPs were successfully prepared by sol-gel method (Sridhar et al. 2016). Indium nitrate/polyvinyl pyrrolidone, In₂O₃/PANI composite nanofibers and pure PANI were investigated to detect Ammonia gas. The In₂O₃/PANI composite exhibited better catalytic response than pure PANI (Nie et al. 2016). PANI, silicotungstic acid and carbon nanotubes based composite material modified glassy carbon electrode was used for amperometric determination of AA (Zhang et al. 2013). Fe₃O₄/PANI/CH biocomposite film was electrodeposited on the surface of carbon paste electrode without using any cross-linker. Laccase used in the work was obtained from *Paraconiothyrium*. The performance of the developed catechol sensor was optimized with respect to material composition, enzyme loading and solution pH. The fabricated biosensor was successfully applied for the amperometric determination of catechol in tea leaf samples (Sadeghi et al. 2015). PANI/Graphene/Cu phthalocyanine nanocomposites modified

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screen-printed electrode was proposed for electrochemical sensing of AA (Pakapongpan et al. 2014).

2.7 PANI and Clays:

Clays exhibited interesting properties and grabbed great attention due to their specific geometries, surface area, electrostatic charge, better processability, colloidal stability and mechanical strength. Clays are finely-grained combination of natural rock or soil material consisting of aluminium phyllosilicates, traces amount of metal oxides (iron, magnesium, alkali metals, alkaline earth metals) and organic matter. Natural clays are hydrophilic in nature and are not compatible with many hydrophobic polymers. The physical blending of clay and polymer may result in separate discrete phases. Such immiscible systems exhibited poor physical interaction between the organic and the inorganic components and poor mechanical/thermal properties. In contrast, polymer/clay nanocomposite has strong interaction and has uniformly dispersed organic and inorganic phases. During the formation of PANI/Clay nanocomposite, polymer intercalates in the inter-lamellar region of clay particles which act as host. PANI/Clay nanocomposites have recently attracted significant interest in scientific research and industrial applications due to its novel electrical and catalytic properties. Some researchers reported that polymerization process is promoted by the catalytic properties of clay (Liu et al. 2007; Pavlidou et al. 2008; Fang et al. 2008; Burrige et al. 2009). The conductivity of the composite varies with the loading amount and nature of clay minerals. In some cases, the conductivity of nanocomposites is slightly lower than pristine polymers and decreases by nano-clay loading, whereas some clay composite exhibited reverse trend (Akbarinezhad et al. 2012, Vijayakumar et al. 2015; Azevedo et al. 2004). EPR and XRD data showed polymerization of aniline on the clay

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surface and inter-gallery regions of the clay (Porter et al. 1997). PANI/Organophilic montmorillonite clay mineral composite modified indium tin oxide electrode was used for the detection of trace levels of Cd^{2+} , Pb^{2+} and Cu^{2+} using square wave anodic stripping (Barros et al. 2015).

The resistivity of PANI/kaolinite composite was investigated in terms of its temperature sensing properties (Duran et al. 2009). Electroanalytical application of Clay based composite materials has been used for the detection of cationic species or organic molecules (i.e., water pollutants and drugs) (Mausty et al. 2004). PANI/Keggin iron clay composite was used for electrochemical detection of ascorbic acid at nano molar level (Lilly et al. 2014). An efficient nanohybrid gas sensor based on PANI/bentonite intercalated composite was used to investigate toxic vapors *viz.* acetone, benzene, ethanol and toluene (Parmanik et al. 2013).

2.8 PANI and Other Synthetic Polymer:

The composites based on conducting polymers along with other insulating synthetic polymers have been studied for rechargeable batteries, conductive coatings, light emitting diodes, gas sensors and antistatic materials (Singh et al. 2007). Aniline and o-Aminobenzoic acid were copolymerized electrochemically by cyclic potential sweep on a microdisk Au electrode in sulphuric acid. The prepared material exhibited an excellent electrocatalytic activity for the oxidation of AA (Xu et al. 1998).

Cu/PANI/Poly-ortho-methoxyaniline Coated Electrodes were applied for comparative analyses for the electrochemical oxidation of ascorbate ions. POMA layers allow ascorbate oxidation reaction at lower over potential. Copper was electrodeposited on PANI/POMA film. Two separate oxidation waves are observed in the case of Cu-PANI whereas a single ascorbate

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oxidation wave and enhanced currents are found in the Cu-POMA (Komsiyiska et al. 2006). PANI modified glassy carbon electrode exhibited good electrocatalytic response for the oxidation of AA (Casella et al. 1997). CdS quantum dots/polystyrene microspheres/GO/PANI based material was used for electrochemical biosensor utilizing K562 cell for signal amplification (Wang et al. 2018). Polypyrrole/PANI modified Au electrode was reported for glutamate sensing using covalent immobilization of glutamate oxidase (Batra et al. 2014). PANI and poly allylamine modified platinum electrode was used for amperometric detection of uric acid. Uriase was immobilized through glutaraldehyde crosslinking and the polymer film electrocatalytically detected peroxide (Wathoni et al. 2014).

PANI/polyacrylic acid films with porous structures were prepared and developed for an enzyme-free H_2O_2 (Hua et al. 2011). Polyacrylic acid acts as a bulky dopant and maintains the charge neutrality of the PANI. PANI/PVB/PS₃ composite material was used to fabricate screen printed pH sensor. The conductivity of a PANI film varies considerably in solutions of different pH and the imine nitrogen sites get protonated, polyvinyl butyral acts as binder and PS₃ as a surfactant (Gill et al. 2007). Ag chloride/PANI/polyvinylpyrrolidone material was used for selective detection of dopamine. The C=O group of PVP form hydrogen bonds with hydroxyl group of AA and prevents its oxidation (Yan et al. 2010). Molecular imprinted polymer/ conducting polymer based hybrid system was used for the electrocatalytic detection of catechol and dopamine (Lakshmi et al. 2009). Amperometric responses demonstrated an excellent selectivity for AA over glucose and dopamine at poly(aniline-co-m-ferrocenylaniline) modified glassy carbon electrode in citrate buffer solution (Chairam et al. 2011). A minimally invasive glucose biosensor capable of continuous monitoring of subcutaneous glucose has been developed using electropolymerized conductive polymer PANI nanofibers as an enzyme

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immobilization material and polyurethane (PU)/Epoxy-enhanced polyurethane (E-PU) bilayer coating as a protective membrane (Fang et al. 2017). PANI/graphite-epoxy composite/alcohol oxidase/horseradish peroxidase was used to detect ethanol (Carolina et al. 2014).

2.9 PANI and Hydro-gel:

Several synthetic and natural polymers have been used as hydrogel. Hydrogels are cross-linked structure of polymers with hydrophilic groups and they swell by absorbing water or other solutes in a process. During swelling a hydrogel maintains its three-dimensional structure and function. Conducting hydrogels are low-cost, lightweight, biocompatible “smart” biomaterials which combine the favorable biocompatibility properties of hydrogels and electrical properties of organic conductors. They exhibited redox switching and good electrical properties, facile small molecule transport, high hydration levels, lack of cell toxicity and lack of susceptibility to infection. Due to simple and effective control over the properties, they have been used as novel bioactive interface.

PANI/hydrogels have been used for numerous biomedical, biotechnological and bioelectronics applications, such as biosensors, tissue scaffolds, drug delivery devices, actuators, artificial muscles, neural probes, cell stimulators, medical electrodes, tissue engineering and electro-controlled drug delivery (Elie et al. 2010; Shi et al. 2016; Tsai et al. 2011; Dou et al. 2016; Shi et al. 2014; Mawad et al. 2012; Green et al. 2010). Many polymers have been used to prepare PANI-hydrogel, such as poly (ethylene glycol) diacrylate, gelatin, methacrylate, poly vinyl alcohol, poly ethylene glycol, polyacrylic acid, polymethacrylic acid, polyacrylamide, poly(N-isopropylacrylamide), cellulose etc (Wu et al. 2015; Wu et al. 2016; Prabhakar et al. 2017; Sharaf et al. 2015).

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Gau et al. reported self-crosslinked PANI hydrogels using oxidative coupling reaction using ammonium persulfate as the oxidizing agent (Gau et al. 2015). During the formation of PANI/polyacrylamide hydrogel, partial PANI chains were grafted on the nitrogen atoms of polyacrylamide. It has a good conductivity of 0.6 S/cm in acidic and neutral conditions due to the formation of highly conductive emeraldine PANI salt (Tang et al. 2008).

PANI hydrogel could adsorb other organic molecules and could be used for affinity biosensors (Yun et al. 2015). PANI hydrogel exhibited excellent conductivity and electrochemical properties and hence could be an attractive material for bioelectronics, immobilization matrix and future-generation energy storage electrodes. It has high surface area and three-dimensional porous nanostructures. The developed glucose sensor exhibited fast response time and superior sensitivity (Pana et al. 2012). Pt-NPs/PANI hydrogel was used for glucose biosensor by immobilizing glucose oxidase. The Pt-NPs effectively catalyzed the decomposition of enzymatically produced H_2O_2 (Zhai et al. 2013). PANI/poly(2-hydroxyethylmethacrylate-co-glycidylmethacrylate) hydrogel was used to immobilize glucoamylase. The amount of enzyme loading depends on medium pH and concentration of enzyme (Bayramoglu et al. 2013).

2.10 PANI and Coordination Complexes:

Generally, the redox center of enzymes is embedded deep inside the protein sheath, which hindered the charge communication between transducer and the redox center. Many small organic and inorganic molecules (e.g. redox dye molecules and transition metal complexes) have been used as redox mediator. Prussian blue has been used as pseudo-peroxidase to catalyze reduction of H_2O_2 and also to detect some other analytes like glutamate, galactose, alcohol,

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fructosyl amine, formate, lysine and oxalate (Ricci et al. 2005). Prussian blue modified Au and platinum screen-printed electrodes were used to develop H_2O_2 amperometric sensors for selective detection (Mattos et al. 2003). Prussian blue/PANI/MWCNTs were used for the fabrication of electrochemical microbiosensor for selective in vivo monitoring of glucose in rat brains. H_2O_2 , generated from the glucose oxidase (GOx)-based enzymatic catalytic reaction was electrocatalytically determined by composite material (Li et al. 2015).

Prussian Blue/PANI/MWCNTs composite film was fabricated by step-by-step electrodeposition on glassy carbon electrode to detect peroxide at an applied potential of 0.0V (Zou et al. 2007). A three dimensional ordered macroporous self-doped PANI/Prussian blue film was fabricated via the inverted crystal template technique using step-by-step electrodeposition. The material was used to fabricate glucose biosensor (Chen et al. 2012). Glucose sensors was fabricated by immobilization of glucose oxidase in a layer of electrochemically deposited PANI over Prussian blue modified platinum electrode (Garjonyte et al. 2000). A CH/Glutaraldehyde crosslinked uricase was immobilized onto Prussian blue NPs absorbed onto PANI/MWCNT-COOH layer, electrochemically over Au electrode. This hybrid electrode was used to determine uric acid in real serum samples (Rawal et al. 2012). PANI/ $CO_3[Fe(CN)_6]_2$ composite material was used for oxygen reduction (Wang et al. 2016).

2.11 Other Sensors:

In addition to electrochemical sensors, some other sensor systems have also been reported, such as pressure sensor (Park et al. 2015), electrical sensor (Bairi et al. 2015), gas sensor based on field effective transistor, (Bai et al. 2007; Lv et al. 2017; Adzhri et al. 2016), chemiresistive sensors (Song et al. 2013), optical sensor (Luo et al. 2017; Omara et al. 2015; Liana et al. 2015),

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mass sensor (Li et al. 2007) and thermal sensor (Hong et al. 2016). A stretchable sensor array to determine pressure was fabricated using Au-deposited polydimethylsiloxane (PDMS) micropillars and the bottom layer of conductive PANI nanofibers on a polyethylene terephthalate substrate (Park et al. 2015). Different hybrid polymer-phthalocyanine materials were prepared by solution processing, using two sulfonated phthalocyanines, s-CoPc and CuTsPc, and polyvinylpyrrolidone, polyethylene glycol, poly(acrylic acid-co-acrylamide), poly(diallyldimethylammonium chloride) and PANI were used as polymer matrix. The sensor response was measured by the change in conductivity of the resistor with respect to concentration of ammonia and relative humidity (Gaudillat et al. 2014).

PANI/PVA was also used to fabricate a microsensor to detect humidity (Yang et al. 2011). A PANI and carboxylated polyvinyl chloride composite was used to develop a conductometric sensor for ammonia (Singh et al. 2008). For detecting ammonia, sensors have also been also reported by the composite materials prepared by insitu chemical polymerization of PANI with polystyrene (Pud et al. 2017), poly(methyl methacrylate) (Zhang et al. 2014), iron-oxide (Bandgar et al. 2015), zinc oxide (Shukla et al. 2012; Shukla et al. 2013) and polyethylene terephthalate (Shukla et al. 2015). A simple room temperature LPG sensing device based on the composite thin film of ZnO/PANI showed 38% sensitivity upon exposure to 4700 ppm of LPG (Kulkarni et al. 2014).

The PANI/Graphene composite was utilized for highly sensitive, reliable and reproducible hydrazine chemical sensor. The sensing performance of the fabricated chemical sensor towards the detection of hydrazine was measured by a two electrode current-voltage (I-V) technique (Ameen et al. 2012). Ag NP/PANI/Tungston phosphate nanocomposite modified glassy carbon electrode was used to detect hydrazine by the I-V (current-voltage) technique

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(Rahman et al. 2016). Tannin doped PANI/TiO₂ composite was synthesized by an in situ polymerization of aniline in the presence of tannin-sulfonic acid and titanium dioxide NPs and it was used to determine ammonia gas (Bairi et al. 2015). A novel ternary composite of TiO₂ nanotubes/PANI/Au NPs was used for photoelectrochemical (PEC) biosensing of L-lactate (Zhu et al. 2015). Antibody (Anti-typhi) was immobilized on TiO₂/PANI for sensitive detection of salmonella antigens from the sample (Sunjay et al. 2016). Paper-based sensors are gaining increasing attention for their potential applications as cost effective point-of-care analysis. Prussian blue/PANI, as an electrochromic indicator on a resistive Au NP, was used to fabricate semi-quantitative paper-based readout systems that can visually measure a change in resistance (Liana et al. 2015).

2.12 Future Directions and Recommendations:

The market for molecular diagnostics is likely to grow at an extraordinary rate over the next 20 years. In recent years, a variety of nanocomposite systems have been used to develop new generation of biosensor technologies. Efforts are going on to develop sensors for routine applications in the near future for pathogen detection, molecular diagnostics, process control, environmental monitoring, food safety and security (Scheller et al. 2014; Lee et al. 2008; Song et al. 2006; Sharma et al. 2009).

This survey indicates that the scientists are exploring novel and superior electrode materials for better electrochemical sensor systems. Composite materials have improved mechanical, electrochemical, optical, electrical properties and there are enough opportunities to tailor the properties in accordance to the desired application. Biocompatible and multifunctional composites promote the stability and integrity of immobilized biocatalysts.

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Future work should concentrate on further clarifying the mechanism of interaction between nanomaterials and biomolecules on the surface of electrodes. Stability of biocatalyst at transducer surface, long term stability of sensor device at normal conditions and self catalyzing electrode materials are some of the important area which need to be explored. A better control over micro/nanostructure and better charge transfer may boost the performance of the sensor. Sensor/biosensor technologies are grooming by integrating the knowledge of the other related subjects e.g. advanced functional nanomaterials, new genomic, proteomic and metabolomic tools, novel molecular biomarkers, advances in mobile communications technology, grid computing to support data computation and hybrid biosensors.

2.13 Conclusion:

We have concluded that PANI based composite materials have been used for variety of technological applications, including sensors/ biosensors. PANI nanocomposite materials with large surface area, high porosity and high conductivity give excellent performance as the electrode material. PANI supports the enzyme immobilization, promotes redox mediation and directs electrochemistry. Blending and composite formation are easy and innovative ways to produce novel materials. Properties of PANI depend upon the ratio of monomer/oxidizing agent, temperature of the reaction and presence of additives. Nature of acid and the associate dopants greatly impact on the morphology, degree of oxidation and protonation and alignment of polymer chains.

PANI composites have improved electrical and electrochemical characteristics, extended charge delocalization, better reaction control, specific nanostructure, easy processing, high surface area, consistency and stability in aqueous dispersions. Numerous types of sensor systems based on PANI based materials have been reported and scientists are continuously working in the

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same direction. They are trying to develop novel materials with superior and advanced properties. Multicomponent advanced functional material with very specific nanostructure is a current trend of research. Scientists are adopting new methodologies to manipulate the physical and chemical characteristics of PANI by using template synthesis, interfacial synthesis, new additive materials, involving biomolecules and functionalize the polymer chains by substitution reactions as a side chain. Proper reaction control and specific tailoring of the properties by developing composite bring novel materials, which possess a set of desired properties for a specific application.