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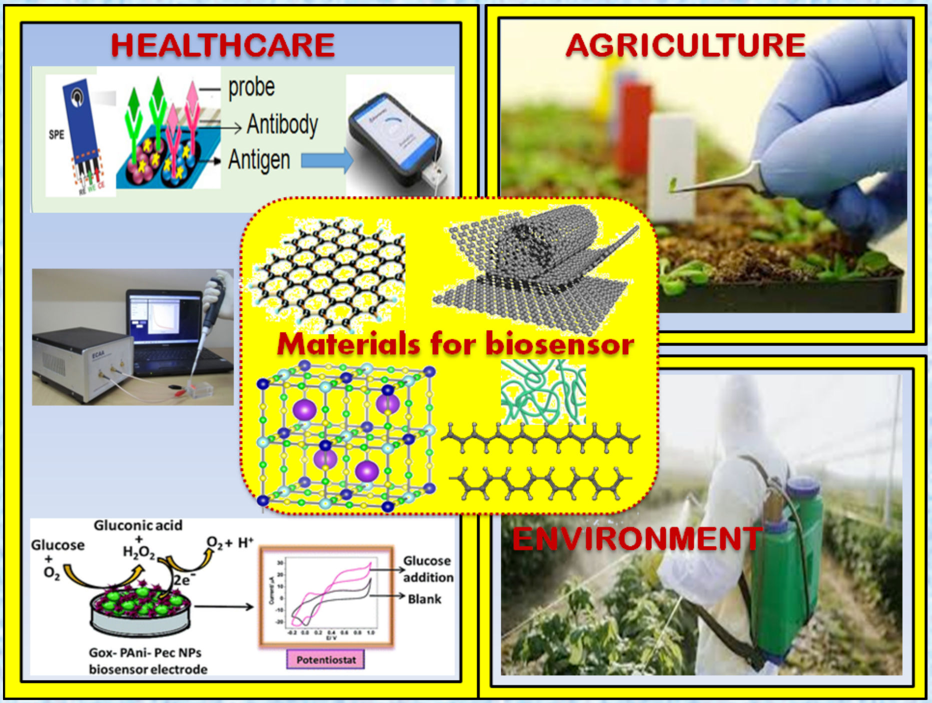

Special Issue on

Materials for Biosensing Applications

SOCIETY FOR MATERIALS CHEMISTRY

Society for Materials Chemistry

Society for Materials Chemistry was mooted in 2007 with following aims and objectives:

(a) to help the advancement, dissemination and application of the knowledge in the field of materials chemistry,

(b) to promote active interaction among all material scientists, bodies, institutions and industries interested in achieving the advancement, dissemination and application of the knowledge of materials chemistry,

(c) to disseminate information in the field of materials chemistry by publication of bulletins, reports, newsletters, journals.

(d) to provide a common platform to young researchers and active scientists by arranging seminars, lectures, workshops, conferences on current research topics in the area of materials chemistry,

(e) to provide financial and other assistance to needy deserving researchers for participation to present their work in symposia, conference, etc.

(f) to provide an incentive by way of cash awards to researchers for best thesis, best paper published in journal/national/international conferences for the advancement of materials chemistry,

(g) to undertake and execute all other acts as mentioned in the constitution of SMC.

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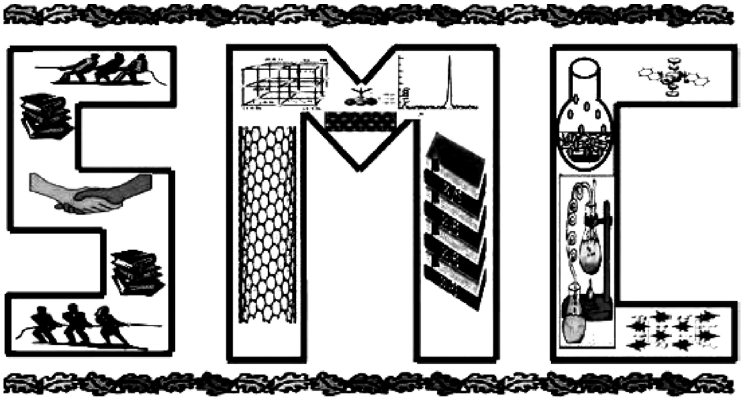

SMC Bulletin

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Guest Editor

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Front cover shows the various nanomaterials and their application in biosensors for Healthcare (detection of cancer biomarker, cancer cells and glucose], Agriculture (detection of minerals and nutrients) and Environment (detection of pesticides).

Guest Editorial

Dr. Shilpa N. Sawant

The growing demand for cost-effective and reliable devices for environmental and health monitoring has led to extensive research on novel sensing approach. Among these, biosensors offer a promising solution for estimation of analytes by exploiting biochemical pathways. Since the first biosensor demonstrated by Clark and Lyons in 1962, the field has grown many folds due to the contribution from multidisciplinary research by chemists, biologist and engineers. The modern-day home blood glucose monitor is one of the most successful example of a biosensor. Biosensors have huge potential for on-field measurements of environmental samples by providing easy to use hand-held or pocket devices. On the other hand, they are being developed in platforms like microfluidic system, lab-on-chip and as flexible devices for wearable biosensors enabling continuous monitoring of essential physiological analytes in healthcare sector. Though biosensor fabrication requires a truly multifaceted approach, development of appropriate sensor materials is one of the most important aspect. Nanomaterials such as carbon nanotubes, graphene, carbon quantum dots, metal nanoparticles, metal-organic frameworks, polymers and biopolymers are extensively utilized for biosensor fabrication. These materials render high sensitivity, stability and reliability to the resulting biosensor. This issue is an attempt to review the contribution of functional nanomaterials towards biosensor development.

This issue of SMC bulletin presents a compilation of articles on materials for biosensors in the area of environmental monitoring, agriculture and healthcare. The role of nanomaterials in signal transduction for biomarker detection in disease diagnosis and toxin monitoring is summarised highlighting some of the recent advancements. The scope of biosensors in agriculture monitoring and for pesticide detection is elaborated right from the concept stage to field application. The articles also cover biosensors being developed in different formats like wearable, flexible, paper based, microfluidics, self-powered biosensors for detection of important metabolites and cancer biomarkers.

It is my pleasure and great honor to serve as the guest editor for this special issue on "Materials for Biosensing Applications". I sincerely thank Dr. A. K. Tyagi, President, SMC and all executive committee members for giving me this opportunity. I express my gratitude to all the authors for contributing their articles at such a short notice and wish to acknowledge Dr Jyoti Korram for contributing to the cover page picture. I hope the readers find the articles engaging and informative.

From the desks of the President and Secretary

Dr. A. K. Tyagi

Dr. Sandeep Nigam

Dear SMC Members, Colleagues and Readers,

Warm greetings from the Executive Council of the Society for Materials Chemistry (SMC)!

Editorial team of the SMC bulletin consistently tries to bring out thematic issues on contemporary interest. All the thematic issues have been multidisciplinary in nature thus bridging the gap between the disciplines of chemistry, physics, biology, and engineering. In the same direction, current issue of the SMC bulletin is also based on materials for biosensing applications.

This thematic issue deal with application of nanomaterials for biosensors in the field of agriculture, environmental monitoring and healthcare. First article discusses the use of nanomaterials in development of electrochemical biosensors for detection of various biomarkers for disease diagnosis and toxin monitoring. Two articles present the utilization of biosensors in the area of pesticide detection and agriculture monitoring at various stages of crop plants starting from soil conditions to the post-harvest and consumption of the agricultural products. Reactive organotrialkoxysilanes mediated synthesis of processable noble metal nanoparticles and Prussian blue nanoparticles has been summarized as an article. The last article presents an overview of biosensors developed in different formats like wearable, flexible, paper based, microfluidics, self-powered biosensors for detection of important metabolites and cancer biomarkers.

We gratefully acknowledge Dr. Shilpa Sawant who agreed to be the guest editor of this issue and put in efforts to bring out this special issue. We also acknowledge the efforts of all the contributing authors for submitting their informative articles. We also thank all the members of SMC for their continued support and cooperation in the growth of the Society for Materials Chemistry.

CONTENTS

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The Art of Signal Transduction: Smart Nanomaterials in Electrochemical Biosensors

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Abstract

In today's fast-paced world, with a desire to obtain everything at the fingertips, electrochemical biosensors have evolved to meet the needs of rapid, onsite, highly sensitive detection of various biomarkers and molecules. The domain of biology has emerged with advancements in technology and is leading to new levels of precision, sensitivity, and specificity in the detection of biomolecules. Development and progress in electrochemical biosensors in the field of diagnosis have led to the utilization of nanomaterials to enhance the sensitivity. Nanomaterials have played a vital role in electrochemical biosensors as detection agents due to their superior electrical, physical and chemical properties and also due to the ability to manipulate and control different features of biological structures for enhanced biomolecular interaction. Presently, the developed electrochemical sensors have integrated several technologies including smart nanomaterials having numerous applications in environmental management, food processing, and disease diagnostics for sensitive, specific, affordable, quick, and point-of-care detection as electrochemical biosensors. This review focuses on the use of nanomaterials in the development of electrochemical biosensors for the detection of various biomarkers/analytes, in the field of disease diagnosis as well as in toxins monitoring. Further, light is also shed on the recent advancements in electrochemical biosensors in wearable technology and its integration with the Internet of Medical Things (IoMT) and also emphasizes electrochemical biosensors challenges and prospects.

Key Words: Smart nanomaterials, electrochemical, biosensors, toxin, point-of-care, wearable sensors

1. Introduction

The emerging field of nanotechnology has set its footprint firm in the advancement of technology ever since the "There's Plenty of Room at the Bottom: An Invitation to Enter a New Field of Physics" lecture was given by physicist Richard Feynman in 1959 [1]. To this day, nanotechnology is still diffusing into several other branches like chemistry, biology, etc., and opening new horizons of applications. Nanomaterials have garnered the attention of researchers due to the enhanced properties they offer when compared to their bulk counterparts. The nanomaterials with a high surface-to-volume ratio provide enhanced opportunities for surface adsorption and exceptional optical, electrical, and chemical properties which are exploited for the development of highly selective, ultra-sensitive biosensors. The use of nanomaterials in the fields of catalysis, nanoelectronics magnetic data storage, biomaterials, biosensors, and structural components are now undergoing quick enhancement [2,3]. Conventional nanomaterials such as gold nanoparticles, iron oxide nanoparticles, graphene, carbon nanotubes, etc., have been reported to have been used in a wide range of applications [4,5]. Recently, gold nanoparticles, carbon nanotubes, gold nanorods [6,7], graphene [8], reduced graphene oxide [9–11] etc., are extensively being used as transducer elements in biosensors. The concept of biosensors dates back to 1962 when it was first published by Clark and Lyons demonstrating the Clark oxygen electrode as the selective transducer for detecting the oxidation of glucose by glucose oxidase (GOx) [12]. Since its conception, the research has advanced exceptionally intending to develop miniaturized, user-friendly, rapid, onsite detection, portable biosensors. Electrochemical detection is extensively used to study molecule interaction, antigen-antibody, aptamer-peptide, enzyme-substrate, etc., recognition. In today's world, electrochemical biosensors are proven to be an important tool in a wide range of applications such as disease monitoring at a personal level, environment monitoring, forensics, etc. Additionally, with the advancement in the field of nanotechnology, the fabrication of electrochemical biosensors has witnessed a breakthrough especially, in developing miniaturized biosensors with increased sensitivity. Figure 1 shows the schematic of electrochemical biosensors with nanomaterials acting as the transducer, and biomolecules as the selector to produce an electrochemical signal on interaction with chemical analytes [13]. This electrochemical interaction signal is captured in an electronic system such as a computer, potentiostat, smartphone, etc. This article focuses on the importance and advantages of various nanomaterials incorporated in electrochemical biosensors and their

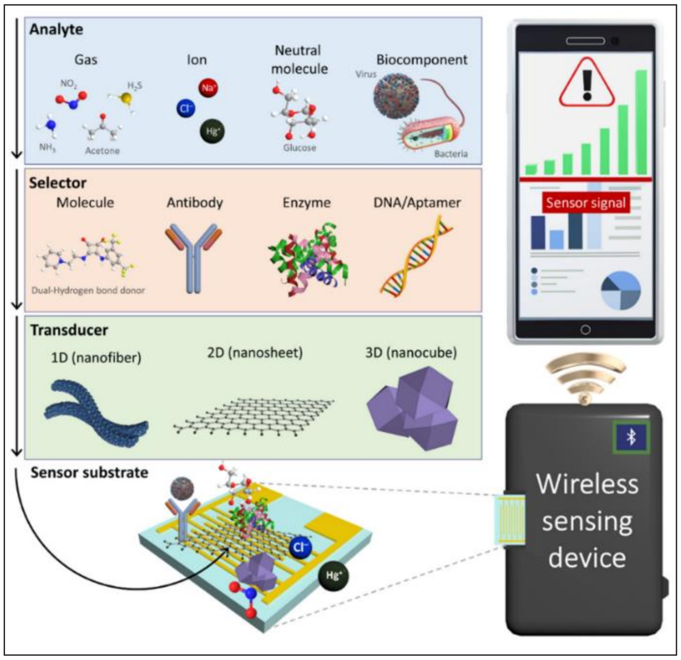

Figure 1: Overview of electrochemical biosensors with nanomaterials as the transducer, and biomolecules as the selector that produce an electrochemical signal on interaction with chemical analytes, captured in the electronic system (Reproduced from Choi et. al., 2022).

application in various fields such as disease diagnosis, toxin monitoring, and wearables.

2. Nanomaterials in Electrochemical Systems

Since the nanomaterial's dimensions are comparable to that of biomolecules, it widens the horizon of research unveiling innovative methods of studying the interaction between the biomolecules such as peptides, aptamers, etc. The combination of enhanced electron transport properties and a high surface-to-volume ratio paves way for the development of smart electrochemical biosensors. Hence the electrochemical biosensors possess the potential of altering their properties in addition to adapting well to their environment as per the requirement of the measuring medium. This enables the fabrication of high-throughput and label-free [14] electrochemical detection systems [15]. Several endeavors focus on the discovery of finding the right nanomaterial to ensure its properties' maximum utilization for the enhancement of the function of the electrochemical system is being undertaken by many researchers. Fundamentally, the material used to make the electrodes must possess high-conducting properties for ensuring the quick transfer of electrons. Fortunately, the field of nanotechnology, especially the emerging sub-field of nanomaterials has set the path for the feasible fabrication of ultrasensitive electrochemical biosensors due to their electrocatalytic activity, high surface area to volume ratio, good biocompatibility, and favorable electronic properties made possible by their nanoscale dimensions. Nanomaterials open up new opportunities for the development of novel electrochemical bioassay systems. Nanomaterials have been utilized to attain the wiring of enzymes directly onto the surface of the electrodes for promoting the electrochemical reactions, imposing barcodes for biomaterials, and also for signal amplification of the biorecognition event [16]. From the literature survey, it is evident that the most widely used nanomaterials in electrochemical systems are carbon-based such as graphene, carbon nanotubes, etc. But recently, other advanced nanomaterials such as metal-organic frameworks have been synthesized and have also been proved their potential application in the electrochemical system and can also be used as carbon alternatives due to enhanced electrical properties. Further, colloidal metallic nanoparticles embedded in the polymeric matrix have also recently gained attention for the development of nanocomposite films [15].

3. Role of Electrochemical Sensors in Disease Diagnosis

The recent COVID-19 pandemic has enlightened the world regarding the importance of rapid, onsite detection of infectious diseases for faster diagnosis and disease management [17,18]. But, due to the ultra-low concentrations of bio-markers at the early stage of infection, the diagnosis of these diseases is challenging [19]. Hence there is a necessity to develop a highly sensitive sensing platform for disease detection. Figure 2 shows electrochemical sensors for the detection of various analytes [20]. In addition to it, in case of life-threatening severe health conditions such as cancer, the early-stage detection of cancer biomarkers give a huge advantage to the patient and also increases the rate of success in reducing cancer progression. To evaluate cancer biomarkers, Chung et al. presented an electrochemical sensing platform using an aptamer that has the ability to change its confirmatory structure. The sensor was used to monitor biomarker therapy response in reducing cancer-related symptoms, low resource settings, and preserving the life of people. Furthermore, to minimize the spread of infectious illnesses that have the ability to become pandemics, identification of the causative agent is essential [21].

In recent years, electrochemical sensors are used to track the effectiveness of therapies and medications by integration of biosensors in-to the nervous system. It is generally understood that Alzheimer's patients have brains with reduced levels of butyrylcholinesterase (BChE). A unique paper-based device was designed by Caratelli et al. to assess the efficacy of medications without any prior

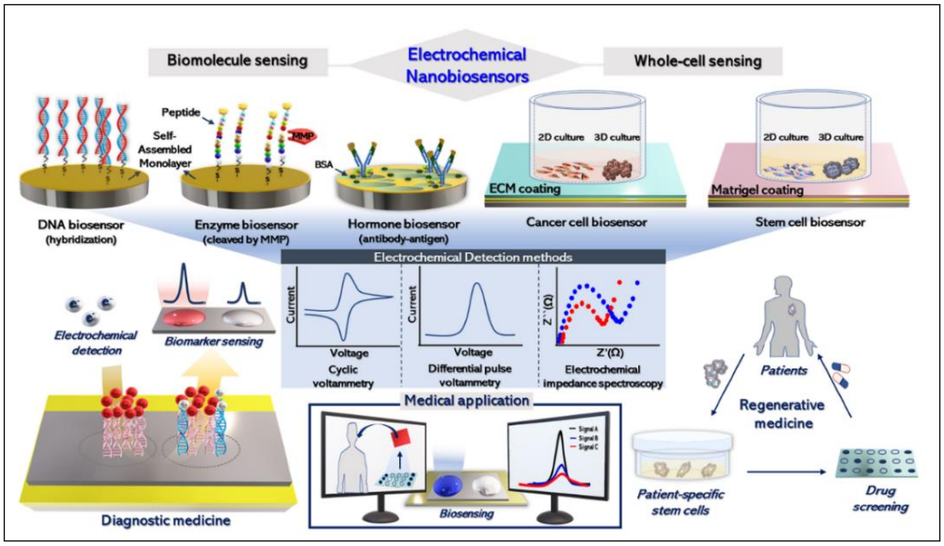

Figure 2: Electrochemical sensors for detection of various biomolecules for disease diagnosis and medicine (Reproduced from Xu et. al., 2020)

pre-treatment by detecting the effects of BChE in the body fluids i.e., blood [22]. DNA-directed immobilization aptamer sensor was also presented by Rycke et al. to find cocaine in sewage water. The sensor was quick in analysis, real-time monitoring, reasonably portable to enable on-site usage, and produced a signal for monitoring environmental samples. Additionally, aspirin, an anti-inflammatory, non-steroidal medicine (NSAID), is frequently utilized to treat fever and decrease inflammation. NSAIDs have been linked to serious side effects including hepatotoxicity, cerebral problems, and renal damage despite their extensive therapeutic use [23]. Diouf et al. presented an electrochemical sensing platform for detecting the role of aspirin in biological fluids inside the body, that is made of chitosan and covered with gold nanoparticles [24]. Therefore, the designing of electrochemical sensors for the accurate assessment of pharmaceuticals is greatly encouraged by these novel sensing concepts.

4. Role of Electrochemical Biosensors in Toxins monitoring

Toxins especially mycotoxins contamination is a serious health concern. It has been reported to be a strong carcinogenic agent and research states that its hazardous to animal and human health and also causes huge economic losses. Thus its detection has been gaining a lot of attention as they are unavoidable contaminants found in human food, animal feed, etc [25–27]. The mycotoxin group of toxins consists of ochratoxin A, T-2 toxin, aflatoxins, etc. [28], and extensive research has been published regarding its detection. These are secondary metabolites produced naturally by fungi. Among the mycotoxins, aflatoxin was reported to be most commonly found and largely produced on food crops when they are stored under room temperatures and humid conditions [29]. Hence, this toxin contaminant is most commonly found in tropical countries and is responsible for the various health hazards among consumers. Further, aflatoxin B1 (AFB1) was stated to be the most carcinogenic and ten times more toxic than potassium cyanide [30]. Thus for the detection of AFB1, Azri et al. have developed a carbon nanotube-based ultrasensitive electrochemical immunosensor (figure 3A). The assay was optimized via indirect competitive enzyme-linked immunosorbent assay (ELISA) and was further moved onto the multi-walled carbon nanotubes/

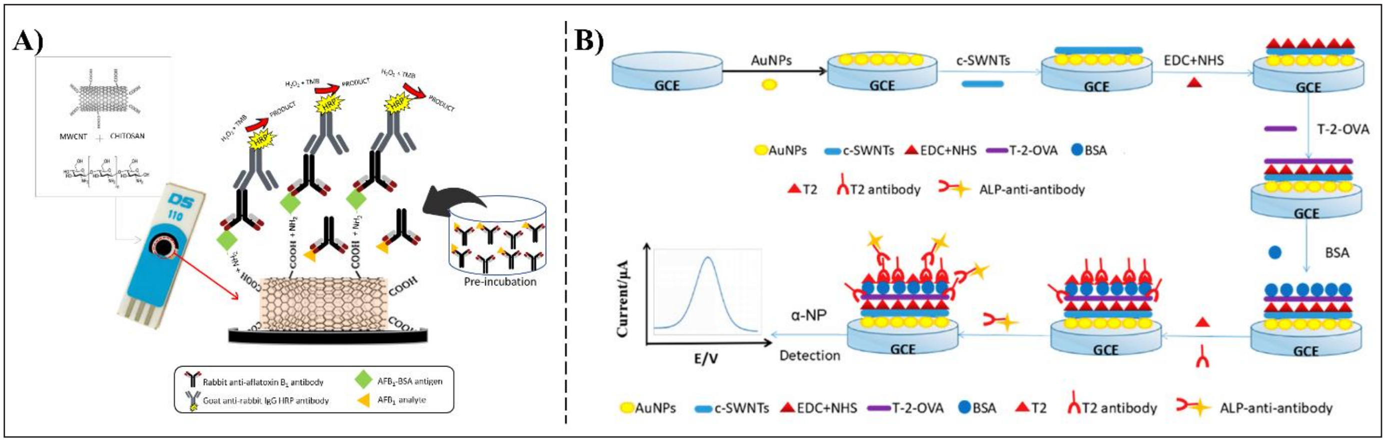

Figure 3: (A) Overview of the nanomaterial-based immunosensor based on ELISA indirect competitive for detection of Aflatoxin-1 (Reproduced from Azri et. al., 2018). (B) The fabrication of Electrochemical Immunosensor Based on Gold-Nanoparticles/ single-walled Carbon Nanotubes/ Chitosan for Sensitive Determination of T-2 Toxin (Reproduced from Wang et. at., 2018).

chitosan/screen-printed carbon electrode (MWCNTs/CS/SPCE). For the detection, Differential pulse voltammetry (DPV) analysis was carried out based on the reduction peak of 3,3′,5,5′-tetramethylbenzidine (TMB) substrate. The sensor LOD was found to be 0.3 pg/mL with a linear working range of 0.0001 to 10 ng/mL. This sensor was also successfully tested on real peanut samples without extra preparation of samples and thus showed potential in quality monitoring of food samples [30]. Another study by Wang et. al., have reported a specific electrochemical immunosensor for the detection of T-2 toxin which is another mycotoxin contaminant. Figure 3B shows the fabrication of the sensor. The immunosensor's electrode is based on gold nanoparticles/carboxylic group-functionalized single-walled carbon nanotubes/chitosan (AuNPs/cSWNTs/CS). Here the hydrolysis of α-naphthyl phosphate substrate catalyzed by alkaline phosphatase produced the electrochemical signal which was detected. This immunosenor was simpler and also more sensitive when compared to other conventional methods of testing. The sensor could detect T-2 concentrations quantitatively 0.01 to 100 μg·L−1 with LOD 0.13 μg·L−1. It also showed an exceptional recovery of 91.42–102.49%. In addition to it, the electrochemical biosensor produced a good correlation with liquid chromatography-tandem mass spectrometry (LC-MS/MS) in the testing of real samples [31].

5. Wearable Electrochemical biosensors for health monitoring

Since time immemorial, the use of plasma and urine samples for the analysis of various metabolites, biomarkers, etc., is the gold standard. However, plasma analysis has its limitations such as the high risk of infection as its invasive nature, the increased cost of total analysis from sample collection to analysis, and the high degree of interference by many plasma proteins in the analysis of specific metabolite/biomolecule. In addition to it, urine sample analysis [32] also comprises limitations as it is laborious, time-consuming, and useful for the detection of analytes with longer retention time in the human body [33]. Hence, the plasma and urine samples are not suitable for dynamic real-time continuous monitoring of biomarkers. All these drawbacks have forced researchers into the creation of wearable sensors for the detection of analytes using body fluids like sweat, tears, saliva, etc., whose samples can be collected non-invasively and continuously monitored [34]. Wearable sensor research has advanced significantly over the last decade, opening up new scientific vistas and providing a basic understanding of engineering and chemistry as they are noninvasive tools for tracking human objectives [35]. Further, sweat, a bodily fluid includes biologically important compounds like proteins minerals and metabolites. As a result, it may signal the state of homeostasis in the body. To improve sweat sampling and sample transport, Li et al. created a flexible circuit board for sweat analysis using paper-based sensing technology [36]. The electronic skin is a network of multimodal sensors that can sense a variety of environmental inputs, including mechanical, physical, and chemical stimuli. For instance, Min et al. created a microfluidic device, flexible in nature that includes a microfluidic layer and a polydimethylsiloxane sensor[37]. This device can improve sweat collecting and provide remote and continuous monitoring of the body's health and performance. Since then, wearable sensors have reportedly improved the accuracy of sweat composition detection. According to Zhang et al. an electronic skin made of cellulose liquid crystal hydrogels with high biodegradability and transmissivity which might be used in flexible electronics in order to further investigate the optical and electrical

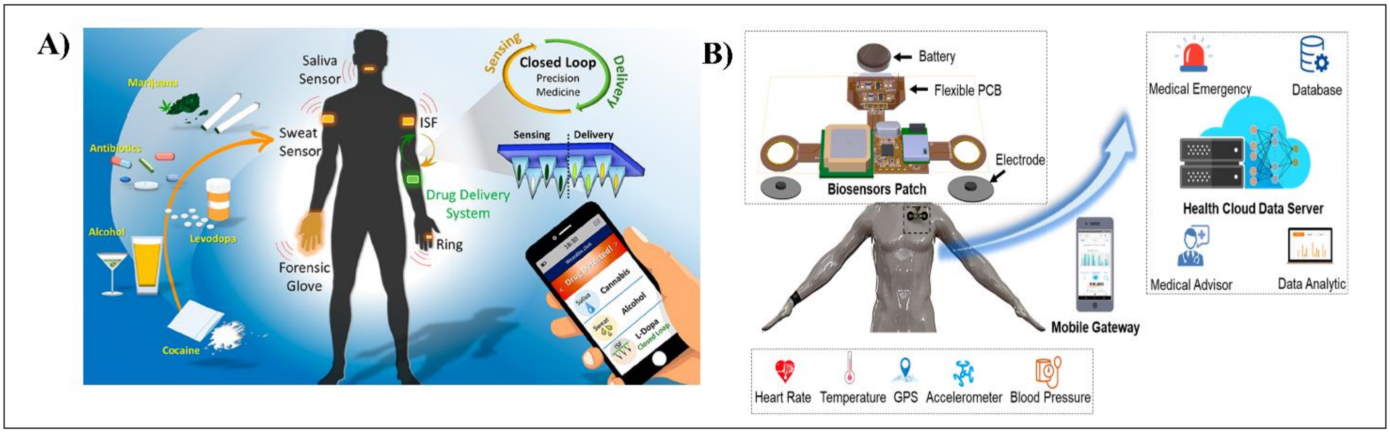

Figure 4: (A) Various analytes detection in wearable electrochemical biosensors with help of smartphone-based application (Reprinted (adapted) with permission from Teymourian et. al., Copyright 2020 American Chemical Society). (B) Overview of wearable biosensor patch with IoMT application (Reproduced from Phan et. al., 2022)

signals of the multifunctional electrochemiluminescence device [38]. Additionally, the skin-attachable gas sensor provides a wearable platform for health monitoring by detecting minute changes in the internal environment. In the future, it is expected that the distinctive manufacture of wearable sensors illustrated above will open the door to the creation of better wearable sensors for electronic skins intended for human-machine interaction.

Moreover, the future holds a treasure trove of opportunities for enhancement in the detection of biomarkers/molecules through wearables. One such future vision is shown in figure 4A [34,39]. It shows the various molecules that can be detected via wearables such as forensic glove, sweat patch, saliva patch, etc. It also shows the possibility of developing a single closed-loop microneedle array capable of continuous real-time sensing of the analyte and also delivering the drugs according to the requirement. Further, this mechanism can also be integrated with the Internet of Medical Things (IoMT) (figure 4B), for improvement in the continuous monitoring and analysis of the molecules. This data can be also shared via the database with various interested personnel such as doctors, family, medical advisors, etc. This data can also be modified with the help of algorithms, artificial intelligence [40], etc., to detect the emergency conditions of patients and intimate the doctor and family members [41].

6. Prospective

This article analyzes the emerging use of smart nanomaterials in the development of electrochemical sensors, including the advantages of nanomaterials over their bulk counterpart, unique electrochemical properties, analytical techniques, and multipurpose applications. Electrochemical technology has advanced in biomedical applications, mainly in disease prognosis, drug testing, and real-time monitoring by developing into an environmentally simple, friendly, and powerful instrument for life healthcare with great simplicity and sensitivity. Although electrochemical biosensing devices have excellent potential, still there is a scope of improvement in further development in this thriving field of research. Firstly, despite the tremendous advancement in the creation of electrical signals using biosensors, the technique for obtaining the target signal still confronts significant difficulties because of its slow response time, dependency on wires, and complicated sample analysis procedures. Advancements in technologies continue to develop, studies have concentrated on wireless power transfer (WPT), bluetooth, and near-field communication (NFC), but there are also easier and simpler ways to conduct electrochemical data transmission without connecting a cable.

Secondly, a crucial factor when employing electrochemical biosensors is the feasible utility and the necessity for wireless connection. Electrochemical workstations have a limited range of applicability since they are often employed in laboratories and are only operated by trained personnel. Therefore, electrochemical instrument miniaturization is highly essential for use by non-trained personnel. In addition to changes in the manufacturing of biosensors, it is essential to incorporate additional cutting-edge innovations, such as cloud connectivity and skin chip technology.

Lastly, even though there are several barriers in the way of electrochemical sensing transition from laboratory research to on-site use, the largest difficulty is its excessive sensitivity. With regard to producing electrochemical sensors with innovative, logically planned capabilities or required mechanical qualities, micromachining technologies have presented a revolutionary approach.

Electrochemical sensors are integrated with smart nanomaterials into several sectors and disciplines. This thorough evaluation is included in this article to make it easier for researchers with backgrounds in material chemistry or biology to quickly examine a variety of smart nanomaterials integrated into electrochemical biosensors.

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References:

1. R. P. Feynman, Eng Sci 23, 22 (1960).
2. A. Kasoju, D. Shahdeo, A. A. Khan, N. S. Shrikrishna, S. Mahari, A. M. Alanazi, M. A. Bhat, J. Giri, and S. Gandhi, Sci Rep 10, 1 (2020).
3. A. Kasoju, N. S. Shrikrishna, D. Shahdeo, A. A. Khan, A. M. Alanazi, and S. Gandhi, RSC Adv 10, 11843 (2020).
4. E. Yasun, S. Gandhi, S. Choudhury, R. Mohammadinejad, F. Benyettou, N. Gozubenli, and H. Arami, J Drug Deliv Sci Technol 60, 102094 (2020).
5. R. Singh Chouhan, I. Jerman, D. Heath, S. Bohm, S. Gandhi, V. Sadhu, S. Baker, M. Horvat, and C. Raghuraj Singh Chouhan, Nano Select 2, 712 (2021).
6. S. Mahari, A. Roberts, and S. Gandhi, Food Chem 390, 133219 (2022).
7. D. Shahdeo, A. Roberts, G. J. Archana, N. S. Shrikrishna, S. Mahari, K. Nagamani, and S. Gandhi, Biosens Bioelectron 212, 114406 (2022).

8. D. Shahdeo, A. Roberts, N. Abbineni, and S. Gandhi, Comprehensive Analytical Chemistry 91, 175 (2020).
9. J. Dey, A. Roberts, S. Mahari, S. Gandhi, and P. P. Tripathi, Front Bioeng Biotechnol 10, 385 (2022).
10. A. Roberts, V. Kesarwani, R. Gupta, and S. Gandhi, Biosens Bioelectron 198, 113837 (2022).
11. S. Shukla, Y. Haldorai, I. Khan, S. M. Kang, C. H. Kwak, S. Gandhi, V. K. Bajpai, Y. S. Huh, and Y. K. Han, Mater. Sci. Eng. C 113, 110916 (2020).
12. L. C. Clark and C. Lyons, Ann N Y Acad Sci 102, 29 (1962).
13. S. H. Choi, J. S. Lee, W. J. Choi, J. W. Seo, and S. J. Choi, Sensors 2022, Vol. 22, Page 610 22, 610 (2022).
14. A. Roberts, S. Mahari, D. Shahdeo, and S. Gandhi, Anal Chim Acta 1188, 339207 (2021).
15. O. A. Sadik, S. K. Mwilu, and A. Aluoch, Electrochim Acta 55, 4287 (2010).
16. D. Andreescu, S. Andreescu, and O. A. Sadik, Comprehensive Analytical Chemistry 44, 285 (2005).
17. A. Roberts, R. S. Chouhan, D. Shahdeo, N. S. Shrikrishna, V. Kesarwani, M. Horvat, and S. Gandhi, Front Immunol 12, 5316 (2021).
18. R. Ahirwar, S. Gandhi, K. Komal, G. Dhaniya, P. P. Tripathi, V. M. Shingatgeri, K. Kumar, J. G. Sharma, and S. Kumar, Biosci Rep 41 (2021).
19. A. Roberts and S. Gandhi, Frontiers in Bioscience - Landmark 25, 1875 (2020).
20. T. Xu, Y. Song, W. Gao, T. Wu, L.-P. Xu, X. Zhang, and S. Wang, Biomedicines 2021, Vol. 9, Page 15 9, 15 (2020).
21. S. Chung, J. K. Sicklick, P. Ray, and D. A. Hall, ACS Sens 6, 1971 (2021).
22. V. Caratelli, A. Ciampaglia, J. Guiducci, G. Sancesario, D. Moscone, and F. Arduini, Biosens Bioelectron 165 (2020).
23. E. de Rycke, C. Stove, P. Dubruel, S. de Saeger, and N. Beloglazova, Biosens Bioelectron 169, 112579 (2020).
24. C. sen Liu, J. Li, and H. Pang, Coord Chem Rev 410 (2020).
25. J. I. Pitt and J. David Miller, J Agric Food Chem 65, 7021 (2017).
26. J. Wen, P. Mu, and Y. Deng, Toxicol Res (Camb) 5, 377 (2016).
27. S. Bräse, A. Encinas, J. Keck, and C. F. Nising, Chem Rev 109, 3903 (2009).
28. J. W. Bennett and M. Klich, Clin Microbiol Rev 16, 497 (2003).
29. L. Afsah-Hejri, S. Jinap, P. Hajeb, S. Radu, and S. Shakibazadeh, Compr Rev Food Sci Food Saf 12, 629 (2013).
30. F. A. Azri, R. Sukor, J. Selamat, F. A. Bakar, N. A. Yusof, and R. Hajian, Toxins 2018, 10, 196 (2018).
31. Y. Wang, L. Zhang, D. Peng, S. Xie, D. Chen, Y. Pan, Y. Tao, and Z. Yuan, International Journal of Molecular Sciences 19, 3895 (2018).
32. P. Mishra, I. Banga, R. Tyagi, T. Munjal, A. Goel, N. Capalash, P. Sharma, C. R. Suri, and S. Gandhi, RSC Adv 8, 23163 (2018).
33. T. Rezaee, R. Fazel-Zarandi, A. Karimi, and A. A. Ensafi, J Pharm Biomed Anal 221, 115026 (2022).
34. H. Teymourian, M. Parrilla, J. R. Sempionatto, N. F. Montiel, A. Barfidokht, R. van Echelpoel, K. de Wael, and J. Wang, ACS Sens 5, 2679 (2020).
35. H. Lu, B. He, and B. Gao, Engineered Regeneration 2, 175 (2021).
36. M. Li, L. Wang, R. Liu, J. Li, Q. Zhang, G. Shi, Y. Li, C. Hou, and H. Wang, Biosens Bioelectron 174 (2021).
37. J. Min, J. R. Sempionatto, H. Teymourian, J. Wang, and W. Gao, Biosens Bioelectron 172 (2021).
38. Z. Zhang, Z. Chen, Y. Wang, and Y. Zhao, Proc Natl Acad Sci U S A 117, 18310 (2020).
39. M. Pravika, J. Jacob, and P. J. K, Biomed Signal Process Control 50, 178 (2019).
40. A. Kaushik, R. Khan, P. Solanki, S. Gandhi, H. Gohel, and Y. K. Mishra, Biosensors, 11, 359 (2021).
41. D. T. Phan, C. H. Nguyen, T. D. P. Nguyen, L. H. Tran, S. Park, J. Choi, B. il Lee, and J. Oh, Biosensors, 12, 139 (2022).

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Biosensors for Pesticides: From concept to the field application

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Abstract

Pesticides are chemicals used in many different sectors such as agriculture, forestry, food industry to kill or control insects, weeds, fungi, rodents and microbes. These pesticides are found to be harmful to human and animal health or to the environment. Accumulation of pesticides in the ecosystem is detrimental if established in higher concentrations for longer periods. Thus, there is need to monitor these pesticides with prompt and accurate analysis. Present article is a brief review of the work carried out in BARC for biosensor detection of pesticides using different materials. Our laboratory has developed microbial biosensors by immobilizing microbial cells on different matrices and associated with different transducers for detection of single to multiple samples of methyl parathion in the laboratory in a very short period of time. The above biosensors use high end costly detection system and can be used in the laboratory only because of its size. Therefore, a handheld optical biosensor device was also designed and developed using the above colorimetric concept and can be utilized for detection of MP directly in the field. Later on, a technology, Biokit was developed for detection of multiple pesticides belong to organophosphate and organocarbamate groups.

1. Introduction:

According to recently released projections from the United Nations, the world's population will cross 8 billion in 2023 and more than half of all people live in just seven countries.[1] China has the world's largest population (1.42 billion), but India (1.41 billion) is expected to claim this title next year.[2] With a present size of 1.41 billion, India currently supports nearly 17.73% of the world population, with 2.4% land resources and 4% of water resources. Keeping pace with these growing numbers, the country will not only have to raise its agricultural production but also the productivity to ensure food and nutrition security of the nation. Rising population has led to increasing the food demand. It is an undisputed fact that the area of the agriculture land on the planet will decrease and the demand for food and fibre for the ever-increasing population will keep on increasing. It is also mentioned that about 25% of produce in agriculture is lost due to insect pests, weeds and diseases. Protection of crop losses is as important as producing food and fibre, and crop protection chemicals have proved their worth in minimizing crop losses during green revolution and are continuing to do so. Pesticides have played an important key role for crop production to control the crop pests such as insects, fungal, weeds and rat etc., and thereby increasing the productivity to meet the food demands and security by decreasing the crop loss from pest. Pesticides had been extensively used worldwide for killing pests in agriculture, household and industry. They may be categorized, considering the targeted pest (pesticides controlling weeds, insects, fungi and rodents may be known as weedicides/ herbicides, insecticides, fungicides and rodenticides respectively) or the chemical composition (viz. organochlorines, organophosphates, pyrethrin, pyrethroids, carbamates etc) or the origin of the pesticide.[3-8]

Due to extensive use of pesticide in agriculture and other allied sector, presence of pesticides and its residues in food commodities and their entry into the food-chain has become a major cause of concern all-over the world. Food safety has become crucial for all involved in the value chain and consumers have to be assured that they are not exposed to an unacceptable level of pesticide residues. Many newspapers, Hindu (10 June 2015 title: Chemical contaminants in household spices) and Deccan Chronicle (8 June 2015 Title: Washing vegetables does not reduce pesticides) had reported the presence of the pesticides in vegetables and spices.[9-10] In 2017, many newspapers such as Hindu and Deccan chronicle on 7th May 2017 reported the presence of pesticides in dried ginger powder.[11-12] Later 18 pesticides were completely banned by Government of India because of the high toxicity concern.[13-14]

Keeping in view of the dietary exposure and risk assessment, The Food Safety and Standard Authority of India (FSSAI) under Ministry of Health and Family Welfare uses the Good Agricultural Practice (GAP) data for fixation of MRL of each pesticide. A central scheme, "Monitoring of Pesticides Residues at National Level" was set up and

NABL accredited laboratories located in different parts of India under the Department of Agriculture, Cooperation & Framers Welfare, Ministry of Agriculture & Farmers Welfare, were participated for collecting and analyzing the food samples of vegetables, fruits, spices, curry leaves, red chilli powder, rice, wheat, pulses, milk, fish/marine, tea, meat, egg and water for retail outlets, APMC markets, mother dairy, organic outlets and farm gate for the possible presence of pesticide residues.[15]

Many traditional analytical methods like HPLC and GC/LC MS-MS have been widely used for pesticide analysis, but they require not only expensive equipment but also highly- trained technicians. Also, these traditional techniques are time consuming and laborious because it requires pre-sample preparation before analysis. Over a course of time, researchers have put efforts to develop promising alternatives for the detection of pesticides which can be used for easy, online and prompt detection with comparable accuracy and sensitivity. Also, approach is such that the sample preparation can be avoided and minimized. In this direction biosensors will play a key role in detection of pesticides.[8]

2. Concept of biosensor for detection of analyte

A biosensor is an analytical device that integrates an immobilized biological element with a transducer to recognize the analyte and the signal due to interaction between analyte and biological element is proportional to the concentration of analyte (Figure 1).

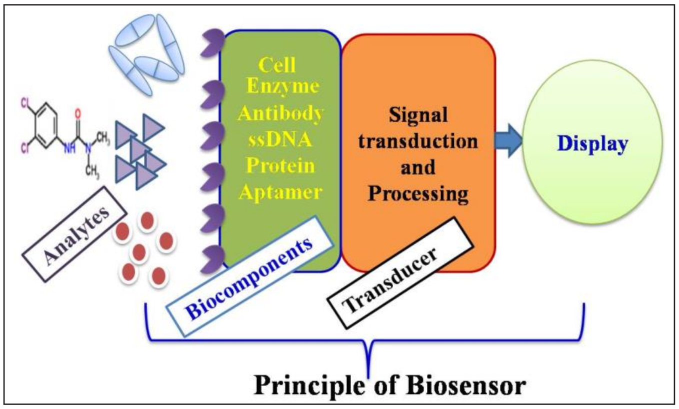


Figure 1. Schematic diagram of principle of biosensor

Biosensor facilitate onsite detection of large number of samples with no or less preparation, less time requirement and no requirement of expensive apparatus and trained personnel which are generally limitation in traditional analytical methods.[15-16]

On the basis of biocomponent, if enzymes are used, it is known as enzymatic biosensor and if microbial cells are used, it is called microbial biosensor. Both biocomponent (enzyme/microbial cells) have certain limitations and advantages. Purified enzymes have very high specificity for their substrates or inhibitors, their application in biosensor construction may be limited by the tedious, time-consuming and costly enzyme purification steps and requirement of cofactor/coenzyme to generate the measurable product. Microbes provide an ideal alternative to these bottle-necks. The enzymes and co-factors that co-exist in the microbes give the ability to consume and hence detect large number of analytes. Microbial cells can be easily manipulated and adapted to consume and degrade new substrates under certain cultivating condition. Therefore, microbial cells are excellent biosensing element for developing biosensors. Thus, using microorganisms as biorecognition element provides an ideal alternative to purified enzyme.

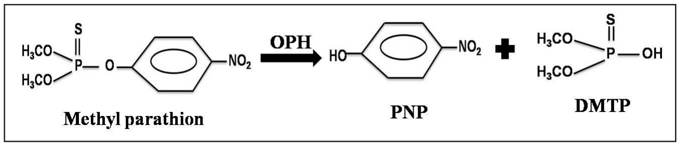
Biosensors require different types of transducer for sensing the signal generated because of interactions of biocomponent with analytes. Electrochemical transducers are widely used in the development of biosensors. According to the detection principle, electrochemical techniques can be divided into potentiometry, amperometry, conductometry and voltammetry. Amperometry is conducted at a given applied potential between the working electrode and the reference electrode and the current signal is recorded between working and counter electrode and correlated to the concentration of MP. Cyclic voltammetry is a very versatile electrochemical technique which allows probing the mechanism of redox and transport properties of a system in solution. This is accomplished with a three-electrode arrangement whereby the potential relative to some *reference* electrode is scanned at a *working* electrode while the resulting current flowing through a *counter* (or *auxiliary*) electrode is monitored in a quiescent solution. The technique is ideally suited for a quick search of redox couples present in a system; once located, it may be characterized by more careful analysis of the cyclic voltammogram. In one of our study, a cyclic voltammetry based microbial biosensor for MP was described. Optical transducer is also commonly used system in biosensors. Optical detection is usually based on the measurement of absorbance, color, luminescence, fluorescence, or any other optical signal produced by the interaction of microorganism with the analyte and correlates the observed optical signal with the concentration of target compound. Optical sensing techniques are especially attractive in high throughput screening since they enable for simultaneous analysis of multiple analytes. The colorimetric sensing technique in microbial biosensors involves the conversion of a chromogen substrate into a colored compound by the metabolic activity of the sensing element. The colored product can be distinguished by the naked eye or a spectrophotometer. Because of its simple and inexpensive measurement setup, colorimetric technique has been widely applied in the fabrication of cost-effective microbial biosensors. Colorimetric biosensors involve the generation of colored compound which can be measured and correlated with the concentration of analyte.[17-21]

3. Biosensors for Methyl parathion pesticides

Methyl parathion (MP) is an organophosphate pesticide which is used as non-systemic insecticide in agriculture to protect the crops. Organophosphates pesticides are a class of insecticides, several of which are highly toxic.[3-5] Earlier they were among the most widely used insecticides however, in the past decade, several notable organophosphates pesticides have been discontinued for use, including parathion, which is no longer registered for any use. Among organophosphate pesticides some widely used pesticides were Methyl parathion, Parathion, Monocrotophos, Chlorpyrifos, Phorate, Profenfos, Quinalphos and Dichlorvos.[8]

3.1. Microbial biosensors for detection of methyl parathion pesticide in laboratory

Among the various biosensors for MP pesticide determination and analysis, systems based on acetylcholinesterase (AChE), organophosphorus hydrolase (OPH) and MP hydrolase (MPH) contribute major share. The basic principle and mechanism of AchE based biosensor is the ability of pesticide to inhibit acetylcholinesterase. On the other hand, OPH and MPH are used based on the enzymatic hydrolysis of MP to generate an acid and alcohol. Below is the structural presentation of MP hydrolysis with OPH into p-nitro phenol (PNP) and dimethyl thiophosphate (DMPT).[3-5]



PNP is an optically detectable product which can be detected by electrochemical and colorimetric methods. Thus, this hydrolytic step has been extensively exploited to develop the biosensor for detection of methyl parathion. MPH also a member of OPH family acts specifically on MP in a similar way. This makes OPH and MPH a suitable recognition element for the detection of methyl parathion pesticide. In this article we will be discussing about the research work carried out in our laboratory for developing the microbial biosensor for monitoring of MP pesticides.[3-5]

Our first study involved an optical microbial biosensor for the detection of MP (Figure 2).

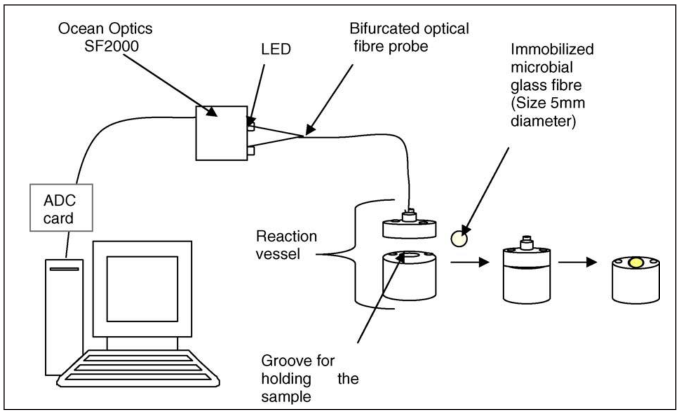


Figure 2. Schematic diagram of optical biosensor using disposable microbial biocomponent

In this study, whole cells of *Flavobacterium sp.* expressing OPH enzyme, were immobilized on glass fiber filters and were used as biocomponent along with an optical fiber system. Detection was based on the relationship between the amount MP hydrolyzed and the amount of chromophoric product PNP formed which was quantified by measuring the absorbance at the λmax of 410 nm. A lower detection limit of 0.3 µM and linear detection range of 4 - 80 µM of MP was established. The immobilized microbial biocomponent was disposable, cost-effective and showed high reproducibility and uniformity. Applicability of biosensor was also demonstrated with synthetic MP spiked samples.[17]

In the second study, recombinant *E. coli* cells with high periplasmic expression of OPH was immobilized on screen printed carbon electrode (SPCE), associated with cyclic voltammetry system and cyclic voltammogram were recorded before and after hydrolysis of MP (Figure 3).[18]

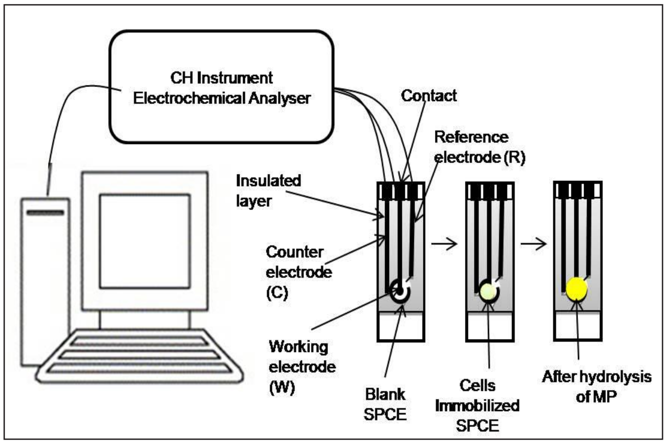


Figure 3. Schematic diagram of electrochemical biosensor using cells immobilized SPCE

It was calibrated based on the relationship between the changes in the current observed at +0.1 V potential. As the concentration of MP was increased the oxidation current also increased. Detection range of biosensor was reported between 2 - 80 µM of MP. A single SPCE with immobilized cells could be reused for 32 reactions and showed storage stability for 22 days.

In the third study, a microplate-based biosensor was described where isolated cells of *Sphingomonas sp.,* were immobilized directly onto the surface of the wells of a polystyrene make 96 wells microplate using glutaraldehyde as the cross-linker (Figure 4).[19]

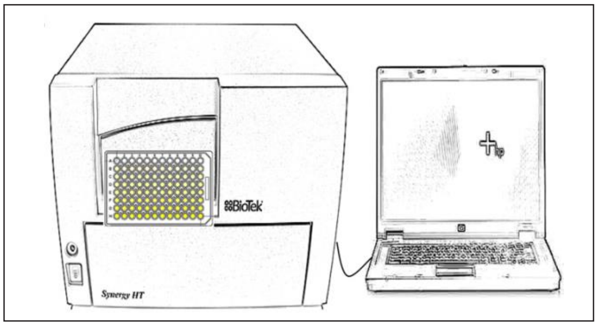


Figure 4. Schematic diagram of microplate based optical biosensor for multiple samples

MP was hydrolyzed to a chromophoric product PNP. Microplate with immobilized bacteria was directly associated with the optical transducer of a microplate reader and PNP was quantified by measuring the absorbance at a λmax of 410 nm. In this case linear detection range of the biosensor was also between 4 - 80 µM MP but the cells-immobilized microplate showed reusability up to 75 reactions and storage stability of 18 days [2, 24]. In another study, inner epidermis of onion bulb scale was used as a natural support for immobilization of microbial cells of *Sphingomonas sp.* In this study, cells immobilized on onion membrane were placed inside the wells of the microplate and same mechanism as mentioned above was used for detection. Detection range was similar because microbial cells and transducer were same but there was a difference in reusability and storage stability which was 52 reaction and 32 days respectively.[20]

In another study, we described the synthesis of a functional biohybrid component by integrating *Sphingomonas* sp. cells with functionalized silica nano particles (^{f}Si NP). Biohybrid was further immobilized onto 96 well microplate for biosensor application. The detection range of this optical biosensor for the detection of MP is 0.1–1 ppm and the storage stability of the biocomponent is 180 days.[21]

All above research works were carried out in our laboratories for establishing the concept of microbial biosensors for the detection of MP pesticides. The characteristic feature of the developed microbial biosensors was the ability to detect MP pesticides in laboratory from single to multiple samples. The first optical microbial biosensor which was developed based on immobilization of whole cells of *Flavobacterium sp.* containing organophosphorus hydrolase enzyme on glass fibre filters dealt with a disposable biocomponent.[17] Biocomponent could be used for single sample analysis only. In the second study, an electrochemical microbial biosensor was developed by immobilizing *recombinant E.coli* on Screen Printed Carbon Electrode (SPCE) and associated with electrochemical analyser.[18] Here the biocomponent was reusable and required low amount of samples. In the third study, a microplate based optical biosensor was developed by immobilizing *Sphingomonas sp.* directly onto the surface of the 96 wells microplate and indirectly on onion membrane fixed inside the wells of microplate and associated with optical transducer of multi detection microplate reader (MDMR). Microplate technique enables to acquire the whole array of data simultaneously and provides an innovative concept where multiple samples could be detected in very short period of time.[19-20] Further integrating *Sphingomonas* sp. cells with ^{f}Si NP increased the sensitivity and stability of biocomponent.[21]

These promising concepts were published in highly reputed journal Biosensors Bioelectronics (Current Impact Factor 12.54). All these microbial biosensors techniques required high end costly detection transducer systems and can be used in laboratory only due to its voluminous size and high cost. Later on, the concept of microbial biosensor exploited and translated into a technology (discussed in next section) for detection of MP pesticides.

3.2. Handheld Biosensors for field detection of MP

BARC has exploited the concept of microbial optical biosensor for detection of MP and translated the research work into technology for developing a prototype of handheld colorimetric biosensor which can be used in the field for monitoring of MP pesticide (Figure 5A and 5B).

It has two components: First component is the biocomponent consisting of immobilized microbial cells of *Sphingomonas* sp. with organophosphorus hydrolase (OPH) enzyme. The second component is the handheld optical colorimeter with an ultraviolet 3W LED light source, a small cuvette and microcontroller circuit.

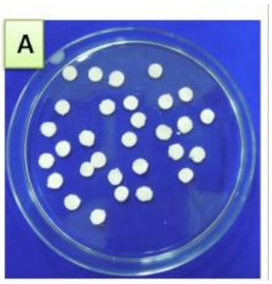


Figure 5A. Biocomponent disc (Microbial cells immobilized) for selectivity

Figure 5B. Handheld colorimetric biosensor for detecting methyl parathion

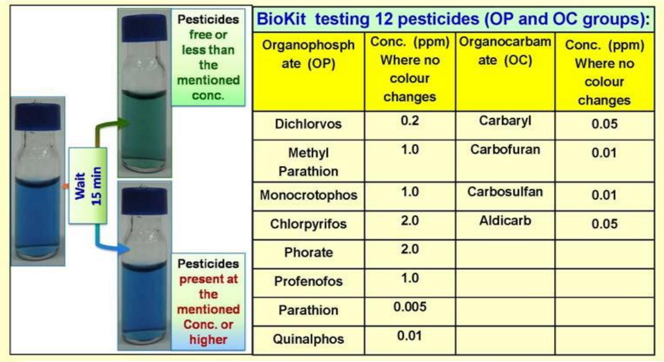
Monochromatic light (410nm) coming from LED passes through glass cuvette (sample in solution) and photodiode for determining the light intensity. This handheld biosensor was initially calibrated with PNP and MP in association with immobilized biocomponent. This handheld biosensor is small in size, battery (DC) operated and directly displays the concentration of MP in ppm (detection range 1-10ppm). This handheld biosensor can be utilized for detection of MP directly in field. This technology is available on our BARC (**AB28NABTD**: http://barc.gov.in/technologies/biosensor/index.html) webpage and has been transferred to entrepreneur in 2018.[22]

4. Biosensor Kit (Biokit) For Detection of Organophosphate and Organocarbamate Pesticides

Using the above technology, one could detect only one pesticide, methyl parathion. However, in real field conditions multiple pesticides are used in large amounts on crops resulting in the accumulation of many pesticides in the fruits and vegetables as well as in water bodies. Organophosphate (OP) and organocarbamate (OC) pesticides are most commonly used pesticides for agriculture and domestic use. Central Insecticide Board and Registration Committee (CIBRC) earlier recommended these pesticides for application in many crops to control the insects. Presence of these insecticides is thus expected in the soil samples, water resources and in food commodities (vegetables, fruits and spices). These pesticides and their residues cause many health problems in humans like overstimulation of muscle and nerve fibers, uncontrollable twitching, convulsions, difficulty in breathing or death due to inhibition of acetylcholinesterase enzyme. In August 2018, the Government of India has banned 18 pesticides which covers many pesticides belong to OP and OC groups. A simple visual detection method for presence of these pesticides in food samples will be useful for farmers, traders and consumers.

BARC has developed a colorimetric visual biosensor kit (BioKit) for detection of safe levels of OP (Methyl parathion, Parathion, Monocrotophos, Chlorpyrifos, Phorate, Profenfos, Quinalphos and Dichlorvos) and OC (Aldicarb, Carbaryl, Carbofuran, Carbosulfan) pesticides (Figure 6.).

This is a qualitative type of detection which indicates the presence of either single or all mentioned pesticides (12 Nos. as mentioned in Table 1) that belong to organophosphate and organocarbamate groups of pesticides in samples. In this Biokit, a colour code, **blue** and **green** was optimized and calibrated with their respective concentration of pesticide (Fig 6.). If pesticides are either absent or present at lesser than the mentioned concentration (Fig 6.) then the colour will change from **blue** to **green** in 15 min. If pesticides are present at higher than the mentioned concentration then there will not be change in colour and **blue** colour will persist. This Biokit is able to detect 6 pesticides among 18 banned pesticides. This technology is available on BARC (**AB37NABTD**: https://www.barc.gov.in/technologies/biokit/index.html) webpage and has been transferred to many entrepreneurs.[23]



BioKit testing 12 pesticides (OP and OC groups):

Organophosphate (OP)	Conc. (ppm) Where no colour changes	Organocarbamate (OC)	Conc. (ppm) Where no colour changes
Dichlorvos	0.2	Carbaryl	0.05
Methyl Parathion	1.0	Carbofuran	0.01
Monocrotophos	1.0	Carbosulfan	0.01
Chlorpyrifos	2.0	Aldicarb	0.05
Phorate	2.0		
Profenofos	1.0		
Parathion	0.005		
Quinalphos	0.01		

Figure 6. Concept of Biokit for detection of OP and OC pesticides

5. Conclusion

In this article, we have summarized the research work carried out in BARC which led the development of biosensor for detection of pesticides. Initial research work was carried out for developing the concept of microbial biosensors for detection of methyl parathion pesticides. Biocomponent was developed by immobilizing microbial cells on various matrices in such a way that it can be used as disposable to reusable components and also transducers were selected so that it can be exploited for single to multiple sample analysis in laboratory. Concept of microbial biosensors was translated into technology (handheld biosensor) for field application in detection of MP pesticide. Using these technologies, one could detect only one pesticide, methyl parathion, but in real field conditions multiple pesticides are used therefore later Biokit was developed for detection of multiple pesticides belong to organophosphate and organocarbamate groups. Further study is going on in laboratory to detect the pesticides belong to other groups and are not covered by these technologies.

6. Acknowledgement:

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7. References

1. https://www.un.org/en/un-chronicle/global-population-will-soon-reach-8-billion-then-what
2. https://www.business-standard.com/article/economy-policy/india-to-overtake-china-as-world-s-most-populated-country-in-2023-un-122071100983_1.html
3. J. Kumar, J.S. Melo, Nova Science Publishers, Inc. (**2015**) pp.89-112 ISBN: 978-1-63463-652-0.
4. J. Kumar, J.S. Melo, *Curr. Trends Biomedical Eng. Biosci., 5(3)* (**2017**) : 555663. DOI: 10.19080/CTBEB.2017.05.555663.
5. J. Kumar, A. Mishra, J.S. Melo, *Austin J. Environ. Toxic., 4(1)* (**2018**): pp.1024 (ISSN: 2472-372X).
6. ATSDR 1999. Atlanta, GA, US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.
7. US EPA. 2003. Case No. 0153. http://www.epa.gov/oppsrrd/REDs/methylparathion.pdf. Signed 05/2003. Accessed 20/11/04.
8. A. Mishra, J. Kumar, J. S. Melo, Bhanu Prakash S, *J. Environ. Chem. Eng.* (**2021**), 9(2) 105067.
9. https://www.deccanchronicle.com/150608/nation-current-affairs/article/washing-vegetables-does-not-remove-pesticides
10. https://www.thehindu.com/todays-paper/chemical-contaminants-in-household-spices/article7300031.ece
11. https://www.deccanchronicle.com/nation/current-affairs/070517/kerala-pesticide-found-in-dried-ginger-powder.html
12. https://www.thehindu.com/news/national/kerala/spices-steeped-in-insecticide-residues/article18404562.ece
13. https://timesofindia.indiatimes.com/india/govt-bans-12-pesticides-with-immediate-effect/articleshow/65408144.cms
14. https://krishijagran.com/industry-news/18-pesticides-banned-by-govt-of-india/
15. J. Kumar and S. F. D'Souza, *HEAT*, Vol. 2 (1) (**2013**) pp.69-75.
16. J. Kumar, *BARC Newsletter*: Founder's Day Special Issue (**2013**).
17. J. Kumar, S.K. Jha and S.F. D'Souza, *Biosens. Bioelectron.* 21 (11) (**2006**) 2100-2105.
18. J. Kumar and S.F. D'Souza, *Biosens. Bioelectron.* 26 (11) (**2011**) 4289-4293.
19. J. Kumar and S.F. D'Souza *Biosens. Bioelectron.*, 26 (4) (**2010**) 1292-1296.
20. J. Kumar and S.F. D'Souza *Biosens. Bioelectron.*, 26 (11) (**2011**) 4399 - 4404.
21. A. Mishra, J. Kumar, J.S. Melo. *Biosens. Bioelectron.*, 87 (**2017**) 332-338.
22. Technology of "Biosensor for Methyl Parathion pesticide" (**AB28NABTD**) (http://barc.gov.in/technologies/biosensor/index.html).
23. Technology of "Biosensor Kit (Biokit) For Detection of Organophosphate and Organocarbamate Pesticides" (**AB37NABTD**) (https://www.barc.gov.in/technologies/biokit/index.html).

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Reactive Organotrialkoxysilanes and their role in designing nanostructured materials for potential biosensing applications

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Abstract

Reactive organic functionalities linked to trialkoxysilane especially 3-aminopropyltrialkoxysilane, [2-[2-(3,4-Epoxycyclohexyl)ethyl]trimethoxysilane, 3-Glycidoxypropyltrimathoxysilane and trimethoxysilane has been demonstrated at one end as an efficient precursors in deriving nanostructured thin film for variety of applications especially in biosensor design and on other hand employed in controlled synthesis of highly stable functional metal nanoparticles and their multimetallic analogues most suitable for exploration in real system of technological significance more specifically meeting variety of requirements from immobilization of sensing components to signal transduction. The current review summarizes their role in: (1) Reactive organotrialkoxysilanes derived biocompatible thin film of organically modified silicate, (2) Reactive organotrialkoxysilanes derived Metal organic framework in nanostructured network of organically modified silicate, (3) Reactive organotrialkoxysilanes mediated synthesis of processable monometallic, bimetallic and trimetallic noble metal nanoparticles, (4) Synthetic insertion of metal nanoparticles and their cheaper transition metal analogues within mesoporous matrix for excitable technical applications, (5) Reactive organotrialkoxysilane derived fluorescent nanoparticles, (6) Reactive organotrialkoxysilane derived functional Prussian blue nanoparticles; with subsequent emphasis on the development of Biosensors technology.

1. Introduction

Developing material by controlling their formation at nanoscale has boosted the introduction of suitable remedy for many unsolved issues. One of such process is Sol-Gel chemistry which offers very unique tools for nanoscale mastering of the materials preparation and directed huge attentions of world scientists during last few decades. In India pioneering work conducted by Late Ram Charan Mehrotra and his group [1] on metal alkoxide has been unique to extend such innovations that subsequently extended the establishment of variety of commercial products and companies at global scenario. Sol-Gel processing associated to hydrolysis, condensation, polycondensation and densityfications of metal alkoxide are the key steps in making such material for variety of technological applications. Apart from variety of such processes metal alkoxide enabled most feasible conditions to undergo sol-gel processing however when metal is replaced by silica, the resulting process allowed the formation of nanostructured silicate with exceptional properties and same is followed in natural material evolution. Accordingly, the introduction of silica in alkoxide yielded the formation of nanostructured domain bridging the gap between physics, chemistry, biology, medial and engineering sciences [2]. Normally the sol-gel processing is triggered either through acid or base catalysis accordingly the role of lewis acid and lewis base character of alkoxide precursors drastically control of precise evolution of silica derived nanostructured material. As a consequence, important functionalities meeting the requirement of lewis acid/lewis base character in alkoxide science has been innovated by synthetic chemists and the introduction of organotrialkoxysilane has been evolved for deriving nanostructured silicate of controlled morphology via controlling the sol-gel processing through the participation of organic functionalities linked to trialkoxysilanes[3]. The presence of suitable functionalities not only control the sol-gel processing of trialkoxysilane however enable specific interaction leading to introduction of specific porous morphology for meeting insitu requirement of innovative technological applications. Four types of such functionalities: 3-aminopropyltrialkoxysilane, [2-(3,4-Epoxycyclohexyl)ethyl]trimethoxysilane, 3-Glycidoxypropyltrimathoxysilane and trimethoxysilane are primarily reviewed and explored in our group for yielding nanostructured domains in variety of formulation and are reviewed wide infra with specific attention on their role in Biosensors design.

2. Reactive Organotrialkoxysilanes derived biocompatible thin film of organically modified silicate

Our research program has been primarily associated on the biosensor design since 1985 onward where requirement of biocompatible matrix for immobilization of sensing components has been one of the major issue associated to the integration of sensing and transduction components of Chemical sensors as shown in Figure-1.

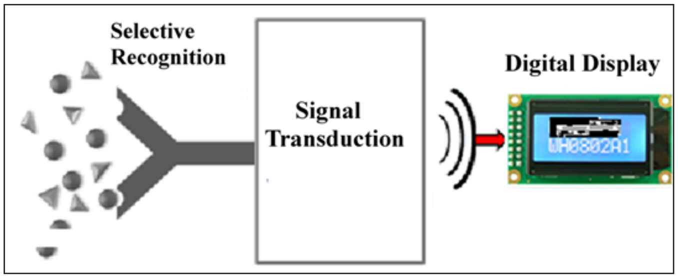

Figure 1 Schematic diagram showing the main components of a biosensor. "Selective recognition" event allow specific interaction of targeted analyte with biocatalyst resulting change in physical parameter sensing component followed by "signal transduction" into "digital display" as a function of analyte concentration.

The integration of sensing component with transduction components determine the success of operation biosensor for practical application. The type of sensing component and transduction unit play central role in integration of these two components. For example while electrochemical mode of signal transduction based on current measurement require the participation of electroactive species that may be either the reaction product are the sensing components itself and specifically three generation of glucose biosensor have been described when electron exchange of glucose and typical glucose oxidase catalysed reaction is considered as given below;

$$\beta\text{-D-glucose} + O_2 \overset{GOD}{\Rightarrow} \text{D-gluocono-1,5-lactone} + H_2O_2$$

Reaction scheme for the first generation glucose biosensor

$$\text{glucose} + \text{GOD[FAD]} \Rightarrow \text{gluconolactone} + \text{GOD[FADH}_2\text{]}$$
$$\text{GOD[FADH}_2\text{]} + \text{Med(ox)} \Rightarrow \text{GOD[FAD]} + \text{Med(red)}$$
$$\text{Med(red)} \Rightarrow \text{Med(ox)} + e^-$$

Reaction scheme for the second generation glucose biosensor.

$$\text{glucose} + \text{GOD[FAD]} \Rightarrow \text{gluconolactone} + \text{GOD[FADH}_2\text{]}$$
$$\text{GOD[FADH}_2\text{]} \overset{\text{[TCNQ-TTF]}}{\Rightarrow} \text{GOD[FAD]}$$

Reaction scheme for the third generation glucose biosensor**.**

Reaction scheme as shown above determine the overall response and selectivity of a biosensor design for commercial applications. In reaction scheme for first generation biosensor, the sensor response is the function of either consumption of oxygen involving oxygen electrode or via oxidation of hydrogen peroxide generated as a function glucose oxidised. Lack of oxygen at the site of enzymatic reaction further directed the development of second generation glucose biosensor where requirement of oxygen is replaced by standard electron transfer mediator and sensor response is dependent on the second order rate constant between redox enzyme glucose oxidase and redox mediator. Certain problems like leaching out of the mediator and other led to introduction of third generation of glucose biosensor where the response of the sensor is the function of the efficiency of the electrocatalyst. Although several membrane materials have been tested including conducting polymer [4,5], organic prepolymer [6] and many more however several of them have their own limitations either associated to leaching out of the sensing components from membrane matrix or morphological variation as a function of time that caused significant alternation in signalling [7-9]. Accordingly, the design of such biocompatible membrane material for encapsulating the same under easily controlled experimental conditions has been challenging research requirement in developing thin film technology. Fortunately, our attentions on these lines has innovated the development of organically modified silicate for encapsulating bacteriorhodopsin protein[10] and further introduced the specific interaction of specific organic functionalities linked to trialkoxysilane. 3- Aminopropyltrimethoxysilane and -[2-(3,4-Epoxycyclohexyl)ethyl]trimethoxysilane were found to be undergo specific interaction based on their Lewis acid and Lewis base character forming polymetric domains of in situ generated morphology as a function targeted sensing component followed by introduction of -Si-O-Si- domains of controlled nanostructured network of organically modified silicate under ambient conditions retaining the biological activity of sensing components [11-13] thereby introducing novel approach of integrating the sensing component together physio-chemical transducer potentially needed in chemical sensors/Biosensors designs. Such nanostructured network ultimately controlled the diffusion of analytes across the sensing components followed by controlling the stability of immobilized components for practical applications. Apart from the specific role of organically modified silicate in immobilizing the sensing components, options to control the nanostructured network that manipulate both analyte diffusion kinetics and electron/ion transfer dynamics could also be manipulated [12,13] by introducing conduction graphite powder and water leachable material within porous network of organically modified silicate. The advantages of such organically modified silicate was not only limited to immobilization of sensing components, controlled transport of analyte and electron hopping sites, the nanostructured domain introduced catalysis during sensing event. Such configuration of modified silicate has really innovated the electrochemical oxidation of hydrogen peroxide in situ generated as a function of oxidase enzyme catalysed reaction at relatively lower over-potential (Figure-1) which normally require high overvoltage on electrochemical oxidation of the same causing major limitations of first generation glucose sensor[11-12].

These finding clearly confirmed the variety of applications of biocompatible nanostructed domains of organically modified silicate especially in the areas: (1) Organically modified silicate based nanostructured domain for chemical sensing, (2) Encapsulation/immobilization of sensing components within organically modified silicate, (3) Manipulation in nanostructured network of organically modified silicate film; which has been innovated and developed at Banaras Hindu University.

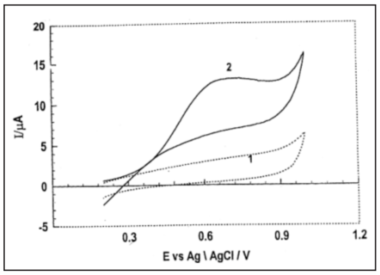

Figure 2 Cyclic voltammograms of glucose oxidase encapsulated within organically modified silicate film in absence and the presence of 500 mM glucose in 0.1. M phosphate buffer pH7.0 at 5 mV/s.

3. Reactive Organotrialkoxysilanes derived Metal organic framework in nanostructured network of organically modified silicate.

Second generation of enzyme electrode are associated with the participation of redox molecules like ferrocene/derivative of ferrocene/TCNQ/TTF and biological sensing component together within immobilized membrane matrix to eliminate the limitations of first generation enzyme electrode [14-23]. When such configuration was tried in organically modified silicate matrix derived from reactive organic functionalities, being one of potent ceramic nanostructured material, electrochemical behaviour was normally associated to sluggish redox electrochemistry (Figure-2A) of encapsulated redox molecules[24]. On the other hand, these materials have already proved as an efficient combination displaying excellent redox bioelectrochemistry that made the introduction of commercial blood glucometer for sensing blood glucose level in a drop of blood sample (video link: https://youtu.be/rVydRLrGOY8). The sluggish electrochemistry was normally associated to the availability of poor electron hopping sited within ceramic matrix and directed us for the search of suitable remedy to manipulate the same for efficient application. Accordingly we intended to fasten the charge transfer process by incorporating conducting components within nanostructured domain of organically modified silicate using graphite microparticles [11-13]. Although such incorporation yielded better electrochemical behaviour of ormosil encapsulated redox moieties however the size of such material which was to the order of 1-50 µ further restricted their use to limited applications. Subsequently, while understanding the role of palladium cations with reactive organic precursors of ormosil like 3-glycidoxypropyltrimehoxysilane and [2-(3,4-Epoxycyclohexyl)ethyl]trimethoxysilane, we observed novel finding on the introduction of palladium nanoparticles within ormosil film based on specific interaction of palladium cations and reactive organic groups like glycidoxy- and epoxycyclohexyl leading to the reduction of the same into palladium nanoparticles [25-31]. These reactive moieties not only allowed the formation of palladium nanoparticles the reduced palladium nanoparticles was subsequently formed –Pd-C- linkage forming palladium linked ormosil. This finding was not limited to such innovative chemistry while dealing with reactive trialkoxysilane as one of the components in ormosil formation, the introduction of -Pd-Si- linkage along with -Pd-C- linkage was introduced within nanostructured network of ormosil converting the nanostructured network of silicate into solid solution that displayed excellent redox electrochemistry of ferrocene monocarboxylic acid better than that recorded in homogeneous solution (Figure-3B) and subsequently allowed the development of commercial blood glucometer with better performance. Trimethoxysilane specifically reacted with palladium chloride forming -Pd-Si- bonding during sol-gel processing of ormosil network. These findings allowed to designs a library of electrocatalytic sites within nanostructured domains for variety of applications [32].

4. Reactive Organotrialkoxysilanes mediated synthesis of processable monometallic, bimetallic and trimetallic noble metal nanoparticles

In ancient times the most spectacular effect of nanoscale material (nanoparticles, NPs) was seen as color pigment in glass and luster industry [33,34]. Metal nanoparticles (MNPs) have been used to color glasses. Lycurgus cup, kept in British museum is one very famous example of nanoparticles used in coloring glasses. In this case gold nanoparticles (AuNPs) have been used to give ruby red color to the cup. Romans manufactured this cup in fourth century, it appears green in daylight and red when illuminated from inside [35,36]. The attention received by MNPs from scientific community can be ascribed to the large surface to volume ratio, intriguing physical and chemical characteristics and surface electronic properties [37].

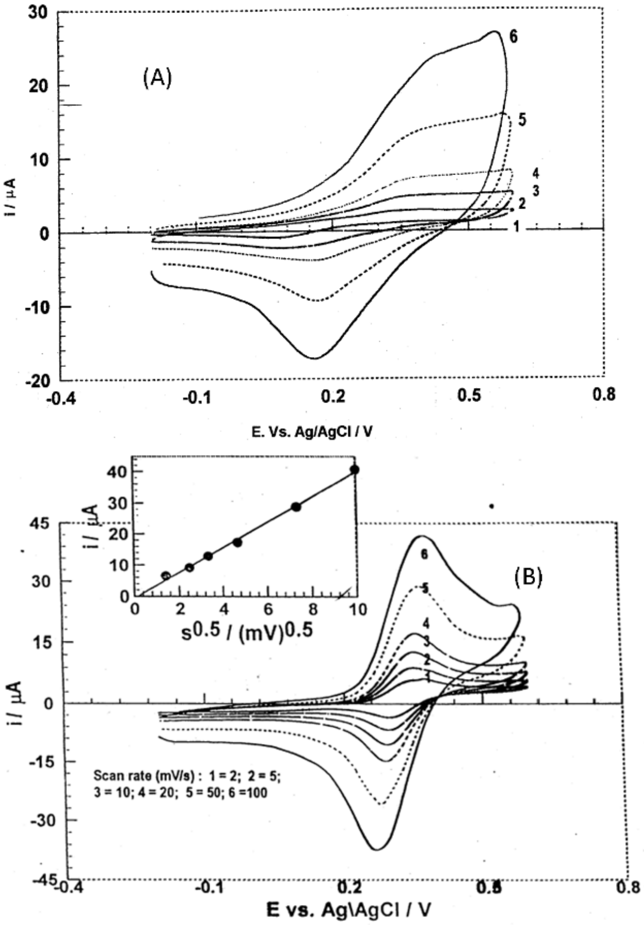

Figure 3: (A) cyclic voltammograms of ferrocence monocarboxylic acid encapsulated within organically modified silicate film at varying scan rate; (B) cyclic voltammograms of ferrocence monocarboxylic acid palladium lined encapsulated within organically modified silicate film ebbedded with -Pd-C- and Pd-Si- network at varying scan rate in 0.1M phosphate bufferpH 7.0.

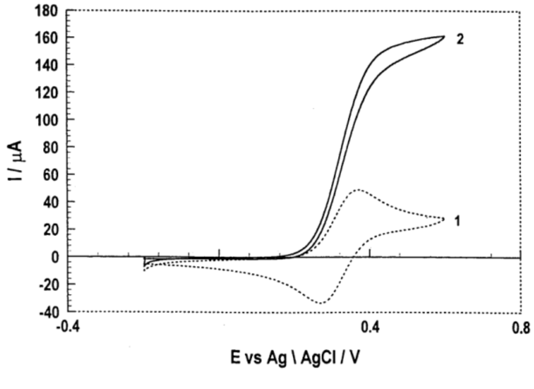

Figure 4 Glucose oxidase catalysed Ferrocene monocarboxylic acid mediated bioelectrochemistry

The use of metal nanoparticles for applications like molecular recognition, biomedicine, energy transfer, sensors and as catalysts is well established [38-40] and further open the excitability in innovating artificial enzyme to design selective biosensor. Noble metal nanoparticles have shown the possibility of mimicking peroxidase enzyme activity. AuNPs have received tremendous attention on these lines because of many important features. First, they exhibit unique size dependent physico-chemical and optical properties. Second, these properties can be tuned by controlling the size, shape and surrounding environment (solvent, ligand etc.). Third, these AuNPs are sub-cellular, non cytotoxic and biocompatible. Fourth, Stable NPs of desired shape can be synthesized easily. One very important property of AuNPs is multifunctionalization that has been exploited for detection of small molecules and biological targets. These finding directed considerable attention of researcher to develop a convenient platform for making nanoparticles most suitable for practical applications in both homogeneous and heterogeneous formulations. Accordingly, considerable efforts have been devoted since last two decades for the synthesis of NPs with a focus on control over its size, shape, dispersibility and functionality. The approaches for the preparation of NPs include both "top-down" and "bottom-up" approaches. After Faraday [41], Turkevisch in 1951 developed a method referred as Turkevisch Method [42] which remains the most popular method for the synthesis of NPs till date due to the ease of synthesis, cheap and non-toxic nature of stabilizer. In this method sodium citrate is used both as stabilizer and reducer. It initially acts as reducer to noble metal cations producing respective nanoparticles and then as stabilizer due to the electrostatic repulsion between the negatively charged oxygen on citrate. The NPs thus produced are spherical with size around 20nm Later, another scientist [43] extended the work of Turkevisch and successfully synthesized variable sizes of NPs by varying the ratio of satabilizer and gold salt. Further, he also noticed that the size of NPs were inversely proportional to the stabilizer concentration i.e. to produce larger particles, less sodium citrate should be added while smaller NPs can be obtained by increasing the concentration of citrate. This method utilizes aqueous medium for the synthesis of NPs hence can be used in aqueous phase only. Subsequently, Brust Method was given by Brust and Schiffrin [44] I to produce NPs in organic solvents that normally form separate layer with aqueous solvent. This method for the synthesis of thiol-protected NPs was a breakthrough in the field of NPs synthesis. It is a two-phase synthesis strategy in which thiol-metal cations interactions are used to protect gold nanoparticles from agglomeration using thiol ligands. Synthesis protocol involves the transfer of AuCl4- from aqueous to toluene media by the use of surfactant tetraoctylammonium bromide and reduced by sodiumborohydride in the presence of octane thiol. A change in color from yellow to deep brown takes place in organic media on addition of sodium borohydride, NaBH4 [44]. The NPs obtained in this case have a size range 1.5-5nm. These NPs exhibit higher stability due to superior Au-thiol interaction. The AuNPs can be further dried and redispersed in organic solvent of choice. The AuNPs obtained in this case show dispersibility in organic phase. Simultaneously, Martin method was developed for the production of "naked AuNPs"[45]. The trick is to stabilize AuNPs, produced by the action of NaBH4 on aqueous HAuCl4 solution, without using stabilizer like citrate or alkanethiols. The NPs produced through this method are monodispersed with diameter ranging from 3.2nm-5.2nm. Martin method is further modified to give octanethiol stabilized AuNPs. The naked NPs obtained through this method can be coated with dodecanethiol and might be phase-transferred to hexane by shaking with mixture of acetone, water and hexane for 30 minutes. Apart from these methods a variety of methods have been explored for making gold nanoparticles using variety of capping and reducing agents. AuNPs can be stabilized by using sodium citrate or by exploiting the strong Au-thiol interactions. While mostly thiol ligands have been used for AuNPs functionalization a variety of other ligands have also been used. Amines are also important as stabilizer because they are omnipresent i.e. present everywhere in biological and environmental system. The first report on the use of amine for AuNPs synthesis was reported[46]. These workers produced hydrophobic AuNPs by using dodecylamine or oleylamine in place of dodecanethiol of Brust-Schiffrin method. Thus, this method is also regarded as a modified type of Brust-Schiffrin method. The precursor made by the interaction between Au3+ and long chain containing primary amine [AuCl(NH2R)] result in AuNPs upon decomposition in air or in tetrahydrofuran [47]. Further, phase transfer technique has been used to generate organically soluble monodispersed AuNPs using amines like Laurylamine and octadecylamine [48]. Oleyl amine [49] aromatic amines [50], amino acids [51] diamines [52], tetraoctylammonium [53], porphyrins [54] and hyperbranched polyethylenimine [55,56] have been used as reducing/capping agents in synthesis of AuNPs, while a direct one-pot synthesis of amine-stabilized AuNPs using 3-(trimethoxysilylpropyl)-diethylenetriamine has been reported [57]. The controlled synthesis of NPs by simple heating in ionic liquid has been developed recently, for which quaternary ammonium-based room-temperature ionic liquids was employed as a solvent, a reducing agent, and as a stabilizer [58]. Piperazine derivatives have also been used as reducing/capping agents during gold nanoparticles synthesis. These outcome certainly direct further insight on synthetic strategy of noble metal nanoparticles, especially on gold nanoparticles synthesis, for real time synthesis and applications.

The conventional routes of NPs synthesis described above have been used exhaustively by scientific community for its application in different fields. These methods have been further modified by the addition of several reagents like PVP, PAP etc. to make the NPs conducive for the concerned applications. However, such techniques for NPs synthesis have following limitations: (i) Generally the AuNPs are produced as aqueous suspension. The use of such NPs in organic solvent causes agglomeration. Similarly the NPs having compatibility with organic solvent (Brust- Schiffrin method) are not compatible in aqueous system. (ii) The NPs fabricated through conventional routes are generally produced as dilute solution. The initial concentration of NPs precursor is very low. (iii) The NPs made through conventional routes are not very stable to changes in pH- and Salt- concentration which limits there use for several applications and the NPs have to be modified to serve the purpose. The NPs made through Turkevisch method agglomerate on addition of single drop of salt. The NPs should be able to adapt to different conditions (change of solvent, change of pH and change of salt concentration) without undergoing any change in its size or shape. (iv) Attempt to convert the homogenous suspension of NPs to heterogeneous matrix by adsorbing over some solid support (TiO_2, Al_2O_3 etc.) causes an increase in size of the NPs i.e. undergo agglomeration. To meet these challenges, sol-gel science and technology, which is most widely accepted methods of creating nanomaterials seems to be more reasonable. Synthesis of sol-gel glasses has received widespread attention because of its applications in various directions [11-13]. Accordingly, the use of several functionalized alkoxysilanes in optimum ratio of hydrophilic character [trimethoxysilane, 3-APTMS] and hydrophobic character 2-(3,4-Epoxycyclohexyl)ethyltrimethoxysilane, 3-glycidoxypropyltrimethoxysilane (3-GPTMS)] have been demonstrated for yielding organically modified thin films for multiple applications[11-13]. Functional alkoxysilanes have been used for casting thin films (through sol-gel processing) that can be used for encapsulating enzymes (glucose oxidase), redox material (ferrocene, ferricyanide etc) and noble metal ions (palladium, ruthenium) and for its use as biosensor . The property of the thin film depends on the concentration and composition of the alkoxysilanes used justified by functional reactivity of the moiety linked to alkoxysilanes while fabricating nanostructured thin films of organically modified silicates through the reduction of palladium cations[25-32]. Some of the functional alkoxysilanes like 3-GPTMS and trimethoxysilane have potential to act as reducing agent for Palladium chloride (PdCl2) that allowed the introduction of Pd within nanostructured network of organically modified silicates[25-32]. Accordingly, such findings directed to examine the role of functional alkoxysilane during the synthesis of noble metal nanoparticles and indeed real time synthesis of all noble metal nanoparticles together their mutimetallic analogues have been made involving the active role 3-APTMS and 3-GPTMS for the first time under ambient conditions[59-62] with further innovation on noble metal nanoparticle synthesis by incorporating microwave exposure of 3-APTMS and 3-GPTMS modulated nanoparticle synthesis with few seconds[63]; [You Tube links: https://youtu.be/Zl-QT574j8Q].The findings reported earlier [59-63] described the use of two functional alkoxysilanes as reducing and stabilizing agents overcoming the limitations of conventional routs of noble metal nanoparticles synthesis and their multimetallic. As disclosed earlier, process was further innovated introducing the role of microwave irradiation that allowed rapid conversion of functional alkoxysilanes assisted formation of noble metal nanoparticles and its multi-metallic analogues [64-72]. These findings precisely demonstrated functional alkoxysilanes assisted controlled and rapid synthesis of noble metal nanoparticles and its multi-metallic analogues. Although the use of functional alkoxysilanes in yielding Ag-NP or other noble metal nanoparticles are most effective however, the Ag-NP formulation that can be converted into: (i) thin film over the surface for biomedical applications like surgical catheter or normal catheter or like, (ii) Ag-NP spray allowing assembling of Ag-NP over any desired substrate just by spraying, (iii) embedding the Ag-NP over the nylon cloth or other cotton cloth are of further potential applications.

5. Reactive Organotrialkoxysilanes derived Siloxane- nanoparticle self assembling nanofluid

A siloxane is the functional group found in organosilicon compounds that contain the Si–O–Si linkage commonly used to create materials for variety of applications. These siloxane polymers are used to prepare surfaces with superhydrophobic properties. Accordingly, a direct synthetic route yielding a colloidal siloxane polymer in a volatile organic solvent would enable facile fabrication of thin membranes with controlled permeability and allowed siloxane polymers to be used as membranes for gas exchange applications (e.g., artificial skin and soft contact lenses) and has been of great attention of advanced research program. Generally, alkylsilane with active participation of –Si-H linkages has been reported for making siloxane polymer. High reactivity of –Si-H functionality restricted the proper exploration of while making siloxane and directed us to make such polymer using reactive organotrialkoxysilane as these precursors allowed controlled synthesis of functional noble metal nanoparticles and their multimetallic analogues. Recently, acetone-induced polymerization of 3-APTMS in chloroform has been observed leading to the formation of siloxane. Using 1H, 13C and 29SiNMR techniques they have proved that acetone reacts with the silane amino group to form an imine [(CH3)2C=N(CH2)3Si(OCH3)3], IPTMS or N-isopropylidene-3-aminopropyltrimethoxysilane. The water that is released during the imine formation hydrolyzes the methoxysilane inducing thereby the formation of siloxane, Si-O-Si bridges. Further, the role of some organic imine, during the synthesis of AuNPs has been documented. Further we have also observed that IPTMS formed from 3-APTMS and acetone as described in our group showed catalytic activity in reduction of noble metal cations into respective nanoparticles and explored during the synthesis of AuNPs, which might be useful in forming AuNPs and siloxane polymer simultaneously given the bifunctional nature of 3-APTMS. Corroborating these findings the synthesis of spherical AuNPs utilizing the interaction between 3-APTMS and acetone has been demonstrated in our group[73-76]. justifying the formation siloxane –nanoparticles nanofluid. Accordingly it is justified that 3-APTMS in several non-aqueous solvent enabled the formation of siloxane and simultaneously enabling the formation of gold nanoparticles having property for casting thin film encapsulating the noble metal nanoparticles. We further demonstrated the controlled polymerization of indole monomers in various organic solvents.[77,78] These findings directed us to understand gold cation-induced polymerization of indole monomer, which forms a Lewis acid–base adduct with gold cations to form a polyindole–gold nanoparticle suspension. This approach may subsequently enable the specific interaction of two colloidal suspensions in acetone, siloxane–gold nanoparticles and the polyindole–gold nanoparticle sol, to yield a polymeric nanofluid of siloxane–polyindole–gold nanoparticles. This type of conversion has been recorded in other volatile solvents such as chloroform, acetonitrile, and dichloromethane. The polyindole–gold nanoparticle suspension underwents self-assembly with the siloxane–gold nanoparticle suspension, yielding a polymer nanofluid that is suitable for making membranes and coatings; the properties of these materials may be tuned with a suitable organic molecule that exhibits the desired functionality. Such material open new horizon on developing extremely stable membrane for biosensor design analogous to that of biological membrane in thickness and also in realizing the analogous interaction of channel forming protein.

6. Synthetic insertion of metal nanoparticles and their cheaper transition metal analogues within mesoporous matrix for excitable technical applications

Porous silica nanoparticles (NPs)/mesoporous silica has been another important materials for variety of application due to their unique properties, such as a large specific surface area and pore volume, controllable particle size and good biocompatibility. Mesoporous silica nanoparticles (MSNPs) with a pore size ranging from 2 nm to 50 nm have been proved as are excellent candidates for drug delivery and biomedical applications. Accordingly, ordered mesoporous silica of M41S family was first reported in the early 1990s,12 followed by subsequent innovations for variety of applications especially in :(1) a large surface area and pore volume for drug adsorption and loading within the pore channels, (2) options for tuning adjustable pore size to control over drug loading and drug release kinetics, (3) for controlled and targeted drug delivery (4) in vivo biosafety, biodegradation, biodistribution and excretion, (5) combinations with magnetic and/or luminescent compounds potentially useful for bioimaging, and (6) synthetic incorporation of metal nanoparticles and their multimetallic analogues for variety of excitable applications ranging from assets for carbon free energy devices, options for achieving tuneable catalytic activity and as bioactive materials for bone regeneration**.** These mesoporous silica are originally derived from tri/tetra-alkoxysilane and proved to be excellent nanomaterial of for further innovations with functional trialkoxysilane derived formulations even before or after achieving controlled mesoporous geometry[79,80]. Accordingly we attempted to to explore the roles of functional trialkoxysilane in both synthetic incorporation of metal nanoparticles and their multimetallic analogues of cheaper transition metals that not only act as support matrix as enzyme mimetic in biosensor application but also serve as vehicle in drug delivery.

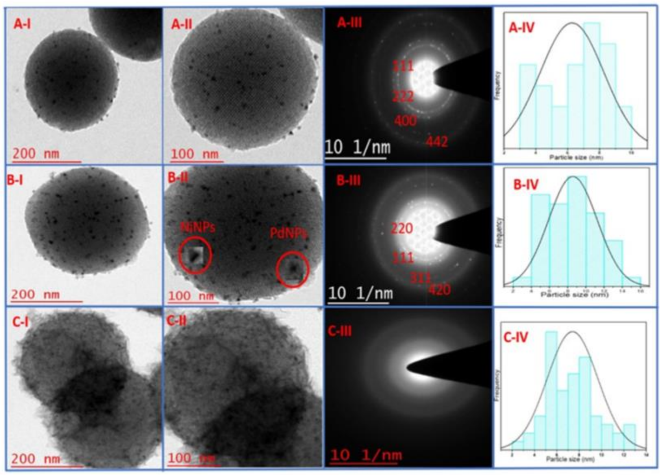

Figure 5 TEM images, SAED pattern and of PdNPs inserted mesoporous silica nanoparticles (A-i-A-iv), Pd-Ni bimetallic [Pd-Ni (1:1 ratio)](B-i-B-iv) and Pd-Ni bimetallic [Pd-Ni(1:2 ratio)] (C-i-C-iv)

7. Reactive Organotrialkoxysilane derived fluorescent nanoparticles.

Generally, Noble metal nanoparticles, especially gold or silver NPs, display weak fluorescence however their use in imaging HeLa cells has been demonstrated[81]. The intrinsic fluorescence of nanoparticles containing metallic gold, silver, and copper has been recorded in recent years [82]. Gold and silver nanoparticles made through conventional rout can be protected using a variety of surface active materials useful in imaging. Various parameters such as particle size, decay times, structural parameters such as ligands on surfaces, metal atom valence states, and crystallinity have been recorded affecting the fluorescence spectra of NPs. Research has been devoted to the development of fluorescent gold nanoparticles that justify real-time fluorescence detection of targeted analytes. The surface functionalization of gold nanoclusters (NCs) with thiol ligands has shown a smart sensing approach towards probing phosphate. The red fluorescence of the gold NCs is quenched by the Eu(III) ions, but fluorescence is restored upon addition of phosphate. Accordingly the role of surface functionalization of gold nanoparticles has been another way to control the fluorescence activity of gold nanoparticles and the role of amine functionalized organotrialkoxysilane have been demonstrated in our group. As described in section 3, 3-GPTMS efficiently allow the reduction of 3-APTMS capped noble metal cations apart from direct reducing ability of 3-GPTMS to palladium cations. The process of nanoparticles formation although has been recorded at room temperature and does not require any additional reagent however the process can be drastically fastened limited to nanoparticles synthesis within minute under microwave exposure of reactive trialkoxysilane mediated formation of noble metal nanoparticles and their multimetallic analogues. It has been found that the gold nanoparticles made in absence and the microwave exposure under each condition are fluorescent and the fluorescence activity can further be controlled by controlling 3-APTMS/3-GPTMS ratio. An increase in 3-GPTMS amount caused reduction in size of gold nanoparticles facilitating the fluorescent activity of the same. In addition the intrinsic fluorescent activity was also found as a function of nanoparticles synthetic strategy whether same is made in absence and the presence of microwave exposure. These finding extended the introduction of sensitive optical sensors for variety of applications since imaging of emission spectroscopy especially fluorescence emission introduce selectivity in analysis. Accordingly, fluorescence spectroscopy played central role in identifying the specific interaction in biomolecules. Our group has been associated to such imaging since 1992 while undergoing the interaction of fluorescence polarization, normally associated to either shift in fluorescence emission as a function of specific electrostatic interaction with the bonding associated to emission spectra. While working on DNA-drug interaction we found that well known fluorescent molecule like Ethidium bromide that bind with DNA [83,84]. Fluorescence polarization has further proved an efficient tool in bio-imaging of antigen antibody interaction when tubulin complexed to metal free phorphyrin like metal free tetrakis(4-carboxyphenyl)porphyrin (TCPP) in the presence of antimitotic agents. Taxol® (paclitaxel), an anti-tumor drug approved for the treatment of a variety of cancers, caused the appearance of a new fluorescence peak at 645 nm at concentrations as low as 125 nM, the intensity of which was a function of the paclitaxel concentration[85] . Cephalomannine caused the induction of a new fluorescence peak at 651 nm only above 1 µM. These findings justified the role of fluorescence polarization in the presence of specific molecule that undergo interaction with bonding of fluorescent molecules[85].

The role of reactive organotrialkoxysilane derived noble metal nanoparticles has been further examined recently by our group while dealing with fluorescence spectroscopy of well-known fluorophores (e.g., fluorescein, rhodamine, and L-tryptophan)[86]. Reactive organotrialkoxysilane, one way protect the nanogeometry of noble metal nanoparticles and on the other hand may also undergo faster electromeric interaction with known fluorescent probe and act as spacer between fluorescent probe and metal nanoparticles. When the concentrations of spacer is altered that ultimately change the distance between metal nanoparticles and spacer followed variation of fluorescence intensity of standard fluorophore either through enhancement or quenching and finally tune either to decrease or increase the nanogeometry of noble metal nanoparticles causing significant variation of emission spectra[86]. Another way of exploring the fluorescence spectroscopy associated to metal nanoparticles in analytical diagnostics is the measurement of resonance Rayleigh scattering under the condition when excitation and emission wavelength are allowed to be same i.e Dλ=0. Many organic analyte has been precisely analysed that alter the RRS signal. Such finding has been demonstrated in non-enzymatic sensing of blood glucose and hydrazine-an organic molecule that can precisely explored in hydrogen evolution. Reactive Organotrialkoxysilane one way allowed synthetic incorporation of Pd-Ni bimetallic within mesoporous silica/silica nanoparticles having efficient catalytic activity for decomposition of hydrazine into hydrogen and nitrogen and otherhand organotrialkoxysilane derived gold nanoparticles acted as efficient nanomaterial for probing the decomposition of the hydrazine based on monitoring RRS signal of organotrialkoxysilane functioned gold nanoparticles[87]. Hydrazine concentration's dependent RRS signal is recorded and were explored in identifying complete decomposition of hydrazine.

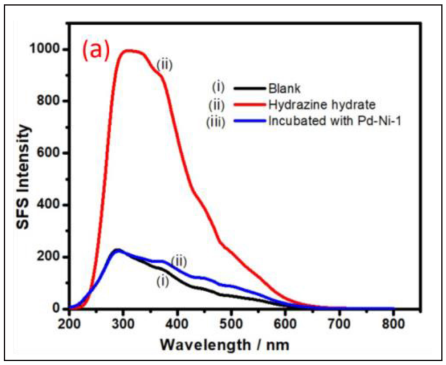

Figure 6 Reactive Organotrialkoxysilane derived function Figure 4. Synchronous fluorescence spectra at delta λ = 0, recorded before (ii) and after (iii) hydrous hydrazine decomposition

Reactive organotrialkoxysilane derived gold nanoparticles has been found to display efficient fluorescence emission and the emission intensity is found as a function of 3-APTMS/3-GPTMA ratio that ultimately controlled the size of gold nanoparticles [88]. Further the emission spectra of as made gold nanoparticles has also been found as a function of excitation wavelength. At the excitation wavelength of 360 nm the fluorescence emission spectra of organotrialkoxysilane functionalized gold nanoparticles is recorded at 460 nm and has been explored in sensitive dopamine sensing in *cerebrospinal fluid* [88]. The nature of emission spectra is also found dependent on the organotroalkoxysilane mediated synthetic strategy whether made under microwave exposure or under ambient condition however, both types of gold nanoparticles are fluorescent. These finding opened a new

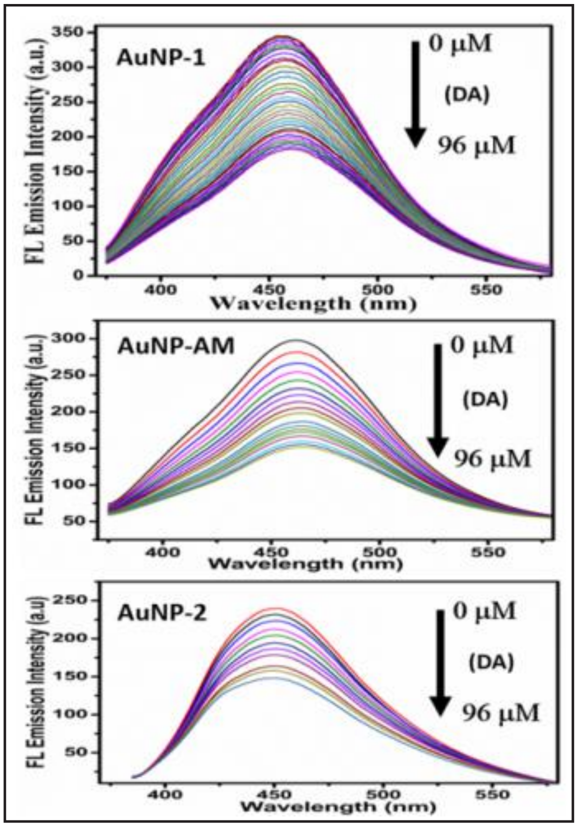

Figure 7 Fluorescence spectra of AuNP made through control over 3-APTMS and 3-GPTMS ratio under microwave exposure (AuNP-1 and AuNP-2) and the same without microwave exposure (AuNP-AM).

rout of sensing and catalytic application in dealing with many unresolved scientific issues.

Metal-hexacyanoferrates (MHCF) have played an important role in variety of scientific applications especially in the field of molecular magnets, as electrode materials in rechargeable battery design, and as electrochromic, ion-exchange, ion-sensing, photomagnetic and electrocatalytic materials. Two metal centers amongst which when both of them is Fe, when coordinates with CN group, a threedimensional framework of cubic symmetry is formed having octahedral coordination and the compounds are known as Prussian blue (PB). Such materials are made either through a co-precipitation method or by electro-synthesis imparting the precise control of the stoichiometric ratio of two metals in the three dimensional frameworks results into rapid nucleation and the PB obtained under this conditions lack functional nanogeometry and processability for specific application thus many unique propertiesof PB remain in question for practical designs. Accordingly, a new synthetic route is required for the synthesis of PB using organic moieties as the active reagents that allow: (i) the simultaneous nucleation of PB, (ii) solubility of the resulting pigment in targetted reaction medium, and (iii) stabilization of the resulting mixed nanogeometry of PB during practical applications. Reactive Organotrialkoxysilane like 3-aminopropyltrimethoxysilane and 2-(3,4-Epoxycyclohexyl)ethyltrimethoxysilane in te presence of cyclohexanone allow controlled synthesis of Prussian blue nanoparticles and their multimetallic analogue for variety of potential applications[89-93].As made Prussian blue nanoparticles behave as peroxidase mimetic and the efficiency of enzymatic like activity can be tuned from the nanogeometry of processable Prussian blue nanoparticles [90-93] potentially useful in biosensor design.

Conclusion

Reactive organotrialkoxysilanes having organic functionalities like alkylamine, epoxycyclohexyl-, glycidoxy- and even trimethoxysilane have been found to create novel nanostructured materials for real applications most suitable for biosensor design as support matrix, electrocatalyst, enzyme mimetics. Our contribution on (1) Reactive organotrialkoxysilanes derived Biocompatible thin film of organically modified silicate, (2) Reactive organotrialkoxysilanes derived Metal organic framework in nanostructured network of organically modified silicate, (3) Reactive organotrialkoxysilanes mediated synthesis of processable monometallic, bimetallic and trimetallic noble metal nanoparticles, (4) Synthetic insertion of metal nanoparticles and their cheaper transition metal analogues within mesoporous matrix for excitable technical applications, (5) Reactive organotrialkoxysilane derived fluorescent nanoparticles, (6) Reactive organotrialkoxysilane derived functional Prussian blue nanoparticles are reviewed justifying their potential in practical applications in biosensor development.

References

1. D. C. Bradley, R. C. Mehrotra and D. P. Gaur, “Metal Alkoxides,” Academic Press, New York, 1978, p. 27.
2. H. SchmidtH. ScholzeA. Kaiser, Principles of hydrolysis and condensation reaction of alkoxysilanes, Journal of Non-Crystalline Solids, 1984, 63, 1-11.
3. A. H. BoonstraC. A. M. Mulder, Effect of hydrolytic polycondensation of tetraethoxysilane on specific surface area of SiO2 gels, Journal of Non-Crystalline SolidsNovember 1988, 105, 201-206
4. Prem C. Pandey, A new conducting polymer-coated glucose sensor, J. Chem. Soc., Faraday Trans. 1, 1988,84, 2259-2265.
5. Prem C. Pandey and A P Mishra, Conducting polymer-coated enzyme microsensor for urea, Analyst, 1988,113, 329-331.
6. C. Tran-Minh, Prem C. Pandey, S. Kumaran, Studies on acetylcholine sensor and its analytical application based on the inhibition of cholinesterase, 1990, 5, 461-471.

7. Y. Degani, and A. Heller, irect electrical communication between chemically modified enzymes and metal electrodes. I. Electron transfer from glucose oxidase to metal electrodes via electron relays, bound covalently to the enzyme, The Journal of Physical Chemistry 1987, 91, 1285-1289.
8. Prem. C. Pandey, C. Tran-Minh, and F. Lantreibecq, Electrochemical Studies on TetrathiafulvaleneTetracyanoquinodimethane modified Acetylcholine/Choline sensor Appl. Biochem. Biotech. 31 (1991) 145-158.
9. Prem. C. Pandey, S. Upadhyay, H. C. Pathak and C. M. D. Pandey, Sensitivity, Selectivity and Reproducibility of Some Mediated Electrochemical Biosensors/sensors, Analytical Letters, 1998, 31, 2327-2348, DOI: 10.1080/00032719808005310
10. Prem C. Pandey , S. Singh, B. Upadhyay, H. H. Weetall and P. K. Chen. Reversal in the kinetics of M-state decay of D96N mutant Bacteriorhodopsin. Sensors & Actuators. B35-36(1996) 270-276.
11. Prem C. Pandey , S. Upadhyay, and H. C. Pathak/ A new glucose Biosensor based on sandwiched configuration of organically modified sol-gel glass. Electroanalysis 11(1999) 59-65.
12. Prem C. Pandey, S. Upadhyay, Ida Tiwari, V.S. Tripathi, n ormosil-based peroxide biosensor – a comparative study on direct electron transport from horseradish peroxidase, Sensors and Actuators B 72 (2001) 224-232
13. Prem C. Pandey , S. Upadhyay & H. C. Pathak. A new glucose biosensor based on encapsulated glucose oxidase within organically modified sol-gel glass. Sensors & Actuators. B60(1999)83-89.
14. Prem C. Pandey, A. M. Kayastha & V. Pandey. An amperometric Biosensor for glucose based on tetracyanoquinodimethane modified graphite paste electrode. Appl. Biochem. Biotech. 1992, 33, 139-147.
15. Prem C. Pandey, S. Glazer & H. H. Weetall. An amperometric flow-injection analysis biosensor for glucose based on graphite paste modified with tetracyanoquinodimethane. Anal. Biochem. 214 (1993) 133-137.
16. Prem C. Pandey, V. Pandey, & S. Mehta. An amperometric enzyme electrode for lactate based on graphite paste modified with tetracyanoquinodimethane Biosensors & Bioelectronics1994, 9, 365- 372.
17. Prem C. Pandey and H. H. Weetall. Application of photochemical reaction in electrochemical detection of DNA Intercalation. Anal. Chem. 1994, 66, 1236-1241.
18. Prem C. Pandey, Tetracyanoquinodimethane mediated flow-injection analysis sensor for NADH coupled with dehydrogenase enzymes Anal. Biochem. 1994, 221, 392-396.
19. Prem C. Pandey, R. W. Aston and H. H. Weetall. Tetracyanoquinodimethane mediated glucose Biosensor based on self-assembling alkanethiol/phospholipid bilayer Biosensors & Bioelectronics, 1995, 10, 669-674.
20. Prem C. Pandey & H. H. Weetall, Peroxidase and Tetracyanoquinodimethane modified graphite paste electrode for the measurement of glucose/glutamate/lactate using enzyme packed bed reactor. Anal. Biochem. , 1995,224, 428-433.
21. J. L. Lima Filho, Prem C. Pandey & H. H. Weetall. An amperometric flow Injection analysis biosensor for sucrose using TCNQ modified graphite paste electrode. Biosensors & Bioelectronics, 1996,1, 169-174.
22. Prem C. Pandey, S. Upadhyay & B. Upadhyay. Peroxide bisensor and mediated electrochemical regeneration of peroxidase. Anal. Biochem., 1997, 252, 136-142.
23. Prem C. Pandey, S. Upadhyay, & H. C. Pathak. Ethanol Bisensor and electrochemical regeneration of NADH. Anal. Biochem., 1998, 260, 195-203.
24. Prem C. Pandey , S. Upadhyay, & H. C. Pathak. A new ferrocene-linked organically modified electrode sol-gel glass and its application in the construction of Ion-selective electrode. Electroanalysis 11(1999) 950-958.
25. Prem C. Pandey, A PROCESS FOR THE PREPARATION OF FERROCENE ENCAPSULATED PALLADIVM-LINKED ORGANICALLY MODFIED SOL-GEL GLASS (PD-ORMOSIL), Indian Patent 196900, 1999.
26. Prem C. Pandey,S. Upadhyay,Ida Tiwari,Soma Sharma, A Novel Ferrocene-Encapsulated Palladium-Linked Ormosil-Based Electrocatalytic Biosensor. The Role of the Reactive Functional Group, Electroanalysis 2001, 13, 1519-1527.
27. Prem C. Pandey and S. Upadhyay, Bioelectrochemistry of glucose oxidase immobilized on

ferrocene encapsulated ormosil modied electrode, Sensors & Actuators 76(2001) 193-198.

28. Prem C. Pandey , S. Upadhyay, Ida Tiwari & V. S. Tripathi, A novel ferrocene encapsulated palladium linked ormosil based electrocatalytic dopamine biosensor, Sensors & Actuators 75(2001) 48-55.
29. Prem C. Pandey , S. Upadhyay and S. Sharma. Functionalized ormosil-based biosensor. Probing a Horseradish peroxidase catalyzed reactions. J. Electrochem. Soc. 150 (2003) H85-H92.
30. Prem C. Pandey , S. Upadhyay, Ida Tiwari & V. S. Tripathi. An Organically Modified Silicate-Based Ethanol Biosensor, Anal. Biochem. 288 (2001)39-45.
31. Pandey, PC & Upadhyay, BC, Role of palladium in the redox electrochemistry of ferrocene monocarboxylic acid encapsulated within ORMOSIL networks, Molecules

2005, 10, 728-39..

32. Prem C Pandey and B. Singh, Library of electrocatalytic sites in nano-structured domains: Electrocatalysis of hydrogen peroxide, Biosensors and Bioelectronics 2008, 24,842-848'
33. P.Colomban,. The use of metal nanoparticles to produce yellow, red and iridescent colour, from bronze age to present times in lustre pottery and glass: solid state chemistry, spectroscopy and nanostructure. Journal of Nano Research, 2009, 8, 109-132.
34. S Philippe. Nanoparticles in Ancient Materials: The Metallic Lustre Decorations of Medieval Ceramics, OPEN ACCESS PEER-REVIEWED CHAPTER, 2012, DOI: 10.5772/34080**.**
35. Leonhardt, U. Invisibility cup. *Nature Photon* 2007, **1,** 207–208.
36. Freestone, I., Meeks, N., Sax, M. & Higgitt, C. The Lycurgus Cup - A Roman nanotechnology. Gold Bulletin, . 2007, 40, 270-277.
37. Marie-Christine Daniel & Didier Astruc, Gold Nanoparticles: Assembly, Supramolecular Chemistry, Quantum-Size-Related Properties, and Applications toward Biology,

Catalysis, and Nanotechnology, Chem. Rev. 2004, 104, 293−346.

38. Otsuka, I, Osaka,M, Sakai, Y., Travelet, C., Putaux, Jean-Luc, & Redouane Borsali, Self-Assembly of Maltoheptaose-block-Polystyrene into Micellar Nanoparticles and Encapsulation of Gold Nanoparticles, *Langmuir* 2013, 29, 49, 15224–15230.
39. Krishnendu Saha, Sarit S. Agasti, Chaekyu Kim, Xiaoning Li, & Vincent M. Rotello, Gold Nanoparticles in Chemical and Biological Sensing, *Chemical Reviews* 2012, 112, 5, 2739-2779
40. Williams, K, Milner, J, Mary D, Kuppan, B, Carl G, Cerniglia E.& Khare,S. Effects of subchronic exposure of silver nanoparticles on intestinal microbiota and gut-associated immune responses in the ileum of Sprague-Dawley rats, Nanotoxicology 2015, 9, 279-289.
41. Thompson, D. Michael Faraday's recognition of ruby gold: the birth of modern nanotechnology. *Gold Bull*, 2007, **40,** 267–269.
42. John Turkevich, Peter Cooper Stevenson & James Hillier, A study of the nucleation and growth processes in the synthesis of colloidal gold, Discuss. Faraday Soc., 1951,11, 55-75.
43. FRENS, G. Controlled Nucleation for the Regulation of the Particle Size in Monodisperse Gold Suspensions. Nature Physical Science 1973, 241, 20–22.
44. M. Brust , M. Walker , D. Bethell , D. J. Schiffrin & R. Whyman , *J. Chem. Soc., Chem. Commun.*, 1994, 801.
45. Matthew N. Martin, James I. Basham†, Paul Chando, & Sang-Kee Eah, Charged Gold Nanoparticles in Non-Polar Solvents: 10-min Synthesis and 2D Self-Assembly, *Langmuir* 2010, 26, 10, 7410–7417.
46. Leff DV, Brandt L, & Heath JR, Synthesis and characterization of hydrophobic, organically-soluble gold nanocrystals functionalized with primary amines. Langmuir 1996,12:4723–4730.
47. Silvia Gomez, Karine Philippot, Vincent Collière, Bruno Chaudret, François Senocqb & Pierre Lecant, Gold nanoparticles from self-assembled gold(i) amine precursors Chem. Commun., 2000, 1945-1946.
48. Anil K, Xu Z, & Xing-JieLian, Gold nanoparticles: Emerging paradigm for targeted drug delivery system, Biotechnology Advances, 2013, 31, 593-606.
49. Aslam, M. Lei Fu, Ming Su, K. Vijayamohanan & Vinayak P. Dravid, Novel one-step synthesis of amine-stabilized aqueous colloidal gold nanoparticles, *J. Mater. Chem.***,** 2004,**14**, 1795-1797.
50. Newman. JDS, & Blanchard, IGJ, Formation of gold nanoparticles using amine reducing agents, Langmuir 2006, 22, 5882-5887.
51. Suresh K. Bhargava, Jamie M. Booth, Sourabh Agrawal, Peter Coloe, & Gopa Kar, Gold Nanoparticle Formation during Bromoaurate Reduction by Amino Acids, Langmuir 2005, 21, 5949–5956.
52. Selvakannan, PR, Senthil KP. More, AS, Shingte, RD, Wadgaonkar,PP, & Sastry, M. One Pot, Spontaneous and Simultaneous Synthesis of Gold Nanoparticles in Aqueous and Nonpolar Organic Solvents Using a Diamine-Containing Oxyethylene Linkage, Langmuir 2004, 20, 295-298.
53. Steven R. Isaacs, Erin C. Cutler, Joon-Seo Park, T. Randall Lee, & Young-Seok Shon, Synthesis of Tetraoctylammonium-Protected Gold Nanoparticles with Improved Stability, Langmuir 2005, 21, 13, 5689–5692.
54. Kotiaho, A., Lahtinen, R., Efimov, A., Lehtivuori, H., Tkachenko, N. V., Kanerva, T., & Lemmetyinen, H. Synthesis and time-resolved fluorescence study of porphyrin-functionalized gold nanoparticles. Journal of Photochemistry and Photobiology, A: Chemistry, 2010, 212, 129-134.
55. Hongwei Duan , & Shuming Nie, Etching colloidal gold nanocrystals with hyperbranched and multivalent polymers: a new route to fluorescent and water-soluble atomic clusters, J. Am. Chem. Soc. 2007, 129, 9, 2412–2413.
56. Shen, Yi, Kuang, Min, Shen, Zhong, Nieberle, J., i Duan, H., & Holger F., Gold Nanoparticles Coated with a Thermosensitive Hyperbranched Polyelectrolyte: Towards Smart Temperature and pH Nanosensors, Angew. Chem.2008, 120, 2259–2262.
57. Haoguo Zhu, Zhengwei Pan Edward W. HagamanChengdu Liang Steven H. & Overbury Sheng Dai, Facile one-pot synthesis of gold nanoparticles stabilized with bifunctional amino/siloxy ligands, J Colloid Interface Sci. 2005, 287, 360-365.
58. Huijuan Bi, Weiping Cai, Caixia Kan, Lide Zhang, D. Martin & F. Träger, Optical spectroscopic studies of the dispersibility of gold nanoparticle solutions, Journal of Applied Physics, 2002, 92, 7491.
59. Prem C. Pandey, D. S. Chauhan. A process for in-situ generation of noble metal nanoparticles and thereafter core shell of the same. Indian Patent 331496, granted on 05/08/2020 and filed on 04/10/2010.
60. Prem C Pandey and D S Chauhan, 3-Glycidoxypropyltrimethoxysilane mediated in situ synthesis of noble metal nanoparticles: application to hydrogen peroxide sensing. Analyst, 137(2012)376-385.
61. Prem C. Pandey, A. K. Pandey and Gunjan Pandey, Functionalized alkoxysilane mediated controlled synthesis of noble metal nanoparticles dispersible inaqueous and non-aqueous medium" J. Nanosci. Nanotechnol. , 14 (2014) 6606-6613.
62. Prem C Pandey and Richa Singh,. Controlled Synthesis of Functional Silver Nanoparticles Dispersible in Aqueous and Non-Aqueous Medium," J. Nanosci. Nanotechnol., 15 (2015) 5749-5759.
63. Prem C. Pandey, M D. Mitra, and R J Narayan,. Organo-tri-alkoxysilane mediated rapid and controlled synthesis metal nanoparticles in both homogeneous and heterogeneous phase and their catalytic applications. MRS Advances 2021, 6, 43–53.
64. Prem C Pandey, D. Pandey and G. Pandey, 3-Aminopropyltrimethoxysialne and organic electron donors mediated synthesis of functional gold nanoparticles and their bioanalytical applications, RSC Advances, 4 (2014) 60563-60573.
65. Prem C Pandey and Richa Singh, Tetrahydrofuran hydroperoxide and 3- Aminopropyltrimethoxysilanemediated controlled synthesis of Pd, Pd-Au, Au-Pd nanoparticles: Role of Palladium nanoparticles on the redox electrochemistry of ferrocenemonocarboxylic acid, Electrochimica Acta, 138 (2014)163-173.
66. Prem C Pandey and Richa Singh,. Controlled Synthesis

of Pd, Pd-Au, nanoparticles; effects of organic amine and silanol groups on the morphology and polycrystallinity of nanomaterials, RSC Advances, 5 (2015) 10964-10973

67. Prem C Pandey, Richa Singh, and Yashawa Pandey. Controlled synthesis of functional Ag, AgAu/AuAg nanoparticles and its nanocomposite with Prussian blue for bioanalytical applications, RSC Advances, 5 (2015) 49671-49679
68. Prem C Pandey, and G. Pandey. Role of organic functionalities linked to alkoxysilane precursors in nanomaterials synthesis and their biomedical applications. MRS Online Proceedings Library 1719, 11–20 (2014). https://doi.org/10.1557/opl.2015.635
69. Prem C. Pandey. Gunjan Pandey, J. Haider and Govind Pandey, Role of organic carbonyl moiety and 3-aminopropyltrimethoxysilane on the synthesis of gold nanoparticles specific to ph and salt tolerance" J. Nanosci. Nanotechnol., , 16 (2016) 6155-6163.
70. Prem C. Pandey, Shubhangi Shukla and Yashashwa Pandey, 3-Aminopropyltrimethoxysilane and graphene oxide/reduced graphene oxide-induced generation of gold nanoparticles and their nanocomposites electrocatalytic and kinetic activity, RSC Advances, 56(2015) 80549-805569.
71. Prem C. Pandeyand Gunjan Pandey, One-pot two-step rapid synthesis of 3- aminopropyltrimethoxysilane mediated highly atalytic Ag@(PdAu) trmetallic nanoparticles, Catal. Sci.Technol., 6(2016) 3911-3917.
72. Prem C. Pandey. Gunjan Pandey, Tunable functionality and nanogeometry in tetrahydrofuran hydroperoxide and 3-aminopropyl-trimethoxysilane mediated synthesis of gold nanoparticles; functional application in glutathione sensing, ***J. Mater. Chem. B***, 2014,**2**, 3383-3390.
73. Prem C. Pandey, G. Pandey. A process for making self-assembled siloxane-polyindole-gold nanoparticle nanocomposite. Indian Patent 343403, granted on 07/02/2020 and filed on 12/09/2017.
74. Prem C. Pandey, G Pandey, G. and A. Walcarius, 3-Aminopropyltrimethoxysilane mediated solvent induced synthesis of gold nanoparticles for biomedical applications. Mater. Sci. Eng. C, 2017, 79, 45.
75. Prem C. Pandey, N, Katiyal, G. Pandey,G, and R J Narayan, Synthesis of self-assembled siloxane–polyindole–gold nanoparticle polymeric nanofluid for biomedical membranes. MRS Communications, 2020, 10, 482–486.
76. A KTiwari, M K, Gupta, G. Pandey, and Prem C. Pandey, Siloxane-Silver nanofluid as potential self-assembling disinfectant: A preliminary study on the role of functional alkoxysilanes, 2023, 4, DOI: https://doi.org/10.37256/nat.4120231576.
77. Prem C. Pandey & R. Prakash. Electrochemical synthesis of polyindole-a study for rechargeable battery application. J. Electrochem. Soc. 145(1998) 999-1003.
78. Prem C. Pandey & R. Prakash. Characterization of electropolymerized polyindole-application in the construction of a solid-state Ion-Selective electrode J. Electrochem. Soc. 145(1998) 4103- 4107.
79. P.C. Pandey, M.D.Mitra, S. Shukla and R. J. Narayan Organotrialkoxysilane-functionalized mesoporousPd–Ni nanocatalyst for selective hydrazine decomposition and sensing. MRS Communications(2021). https://doi.org/10.1557/s43579-021-00018-y
80. Prem C. Pandey, Murli Dhar Mitra, Atul Kumar Tiwari and Shwarnima Singh, Synthetic incorporation of palladium-nickel bimetallic nanoparticles within mesoporous silica/silica nanoparticles as efficient and cheaper catalyst for both cationic and anionic dyes degradation, 2021, 56, 460-472.
81. H. He, C. Xie, J. Ren, Nonbleaching fluorescence of gold nanoparticles and its applications in cancer cell imaging, Anal. Chem. 80 (15) (2008) 5951–5957.
82. Y. Lu, W. Chen, Sub-nanometre sized metal clusters: from synthetic challenges to the unique property discoveries, Chem. Soc. Rev. 41 (9) (2012) 3594–3623.
83. Prem C. Pandey & H. H. Weetall. Detection of aromatic hydrocarbon based on DNA Intercalation using an Evanescent wave biosensor. Anal. Chem. 67 (1995) 787-792.
84. Prem C. Pandey, & H. H. Weetall. Evanescent wave Fluorobiosensor for the detection of polyaromatic hydrocarbon based on DNA intercalation. Appl. Biochem. Biotech. 55 (1995) 87-94.
85. Sergi Morais, Prem C. Pandey, W. Chen and Ashok Mulchandani A novel bioassay for screening andquantification of Taxanes, Chem. Commun. 2003, 1188-1189
86. Prem C Pandey, S. Shukla, G. Pandey and Roger J. Narayan Organotrialkoxysilane-mediated controlled synthesis of noble metal nanoparticles and their impact on selective fluorescence enhancement and quenching, J. Vac. Sci. Technol. (2020). B 38, 052801.
87. Prem C. Pandey, M. D. Mitra, S. Shukla, and R J Narayan. Organotrialkoxysilane-functionalized mesoporous Pd–Ni nanocatalyst for selective hydrazine decomposition and sensing. MRS Communications, 2021, 11, 78–85.
88. Murli Dhar Mitra , Prem C. Pandey, Functional trialkoxysilane mediated controlled synthesis of fluorescent gold nanoparticles and fluoremetric sensing of dopamine, Optical Materials 2022, 132, 112810.
89. Prem C.Pandey D.S.Chauhan and Ashish K Pandey "Novel synthesis of Prussian blue nanoparticles and nanocomposite sol: Electro-analytical application in hydrogen peroxide sensing" Electrochimica Acta, 87 (2013)1-8.
90. Prem C Pandey and Ashish K Pandey, "Cyclohexanone and 3-aminopropyltrimethoxysilane mediated controlled synthesis of mixed nickel-iron hexacyanoferrate nanosol for selective sensing of glutathione and hydrogen peroxide"Analyst, 138(2013)952-959.
91. Prem C. Pandey, S. Singh, Shilpa N Sawant. Functional alkoxysilane mediated controlled synthesis of Prussian blue nanoparticles, enablin g silica alginate bead development; nanomaterial for selective electrochemical sensing. Electrochimica Acta, 287 (2018)37-48. o Prem C. Pandey , S. Singh, A. Walcarius, Palladium-Prussian blue nanoparticles; as homogeneous and heterogeneous electrocatalysts Electroanal.Chem., 780(2018)747-754.
92. Prem C. Pandey, S. Shukla and R. J. Narayan. Organotrialkoxysilane-Functionalized Prussian Blue Nanoparticles-Mediated Fluorescence Sensing of Arsenic(III). Nanomaterials 2021, 11, 1145. https://doi.org/10.3390/nano11051145

93. Prem C. Pandey, H. P. Yadav, S. Shukla and R. J. Narayan, Electrochemical Sensing and Removal of Cesium from Water Using Prussian Blue Nanoparticle-Modified Screen-Printed Electrodes, Chemosensors 2021, 9, 253.https://doi.org/10.3390/chemosensors9090253.

	*Prem Chandra Pandey, Professor (HAG): https://www.iitbhu.ac.in/dept/chy/people/pcpandeyapc ; http://sensors-vns.com/asset/pdf/Prof.%20P.%20C.%20Pandey.pdf: received M.Sc. and Ph.D. in Chemistry from Gorakhpur University. After completing Ph.D. work in the area of Applied Electrochemistry, Prof Pandey has been associated with the development of Biosensors technology from 1985 onward. Published 200 publications and 55 patents on his own credit supported by youtube videos (https://studio.youtube.com/channel/UCb7rZ26MzeP9m_a-KzvC1DQ/videos) and established company for the commercialization of research outcome in Varanasi [https://sensors-vns.com/ ; http://www.sensors-vns-opc.com/. Listed in top 2% scientist of the world as single Analytical Chemist from Uttar Pradesh.*

Scope of Biosensors in Agriculture Monitoring

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Abstract

A biosensor is a device that helps us to analyse things better. It converts biological signals into electrical signals. It basically comprises of biological element and physiochemical detectors. Agriculture is a complex but systematic practice which includes various process, techniques, methods and technologies, that lead the produce farm to fork. The process of augmenting crop production has made the agricultural ecosystems to face a lot of challenges. This article is a brief review of scope of biosensors in the field of agriculture. Biosensors have many applications at many stages of crop plants starting from soil conditions to the post-harvest and consumption of the agricultural products.

1. Introduction

1.1. Biosensor concept

A biosensor is an analytical device that integrates an immobilized biological element with a transducer to recognize the analyte and the signal due to interaction between analyte and biological element is proportional to the concentration of analyte (Figure 1).

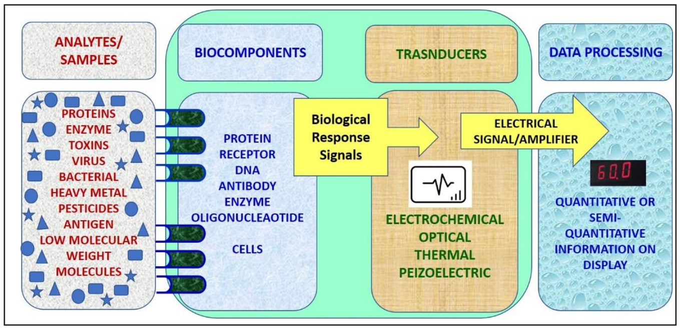

Figure 1. Schematic diagram of principle of biosensor

A physicochemical transducer or transducing microsystems that are either closely linked to or incorporated inside a biological material, a material derived from biology, or a biomimic are used as the recognition molecules in biosensors. Producing a digital electrical signal that is proportionate to the concentration of a particular analyte or collection of analytes is often the goal. Biosensor facilitate onsite detection of large number of samples with no or less preparation, less time requirement and no requirement of expensive apparatus and trained personnel which are generally limitation in traditional analytical methods.

1.2. Types of Biosensors

The pioneers named Clarke and Lyons began Biosensor in late 1960s. Different kinds of biosensors being utilized based on two elements namely known as sensing element and transduction modes (Figure 2).

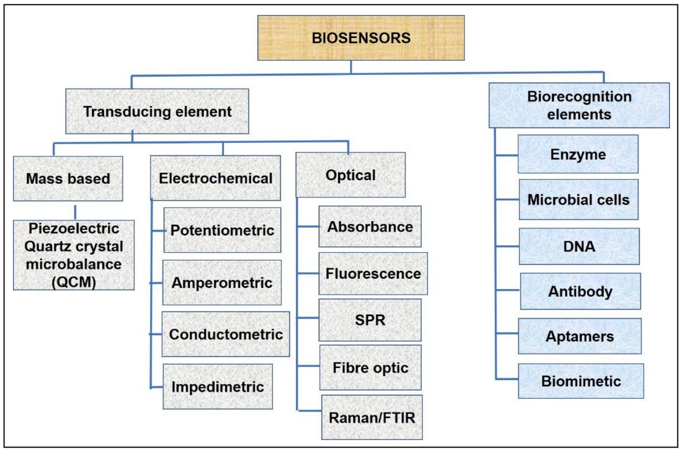

Figure 2. *Types of Biosensors*

Enzymes based biosensor, immunosensor which includes antibodies, DNA biosensor, Thermal and piezoelectric biosensor, biological tissues, organelles and microorganisms which can be detected with the help of whole cell biosensor comes under the category of sensing element. Transduction mode relies upon the physiochemical change coming about because of detecting component. Subsequently on the premise of various transducers, biosensors can be electrochemical (amperometric, conductometric and potentiometric), optical (absorbance, fluorescence and chemiluminescence), piezoelectric (acoustic and ultrasonic) what's more, calorimetric.[1-2]

2. Scope of Biosensors in Agriculture monitoring

Agriculture is a complex but systematic practice which includes various process, techniques, methods and technologies, that lead the produce farm to fork. In agriculture among the various crop stages, pre-harvest stages such as soil conditions as well as when crops in the field and post-harvest stages are the broadly classified stages. Biosensors have many applications at many stages of crop plants starting from soil conditions to the post-harvest and consumption of the agricultural products (Figure3). Many review have very well reported in details about the biosensors application in agriculture (Table 1).

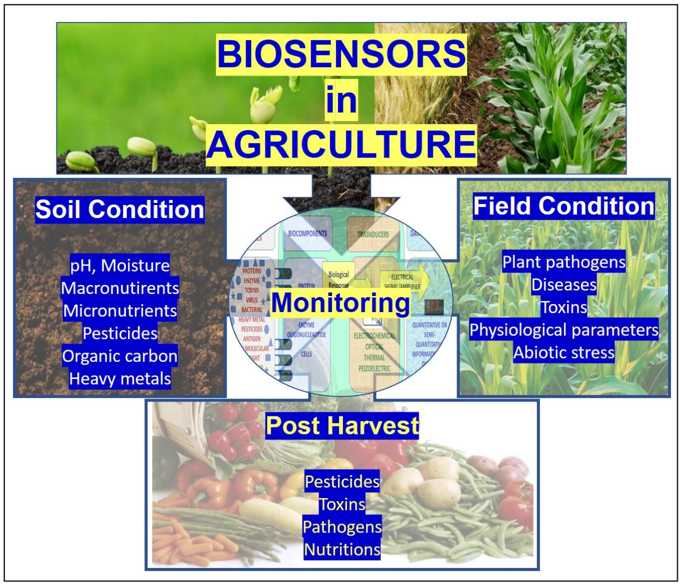

Figure 3. Scope of biosensors in agriculture monitoring

Before showing the seeds, there is need to check the soils conditions such as soil pH, moistures, macronutrients, micronutrients, pesticides and organic carbon etc to get the exact information about the soils and their requirements in terms of inputs. Another analytes in soil is the persistence of lethal heavy metals comprising cadmium, mercury, copper, zinc, nickel, lead, and chromium as they are held responsible for prolonged and significant damage to various physiological function of the plants by disrupting the biological actions such as disruption of photosynthesis and mineral absorption, interruption of electron transport chain, induction of lipid peroxidation, disturbance in the metabolism of essential elements, induction of oxidative stress and by damaging the plant organs like root, leaves, and other cellular components. Moreover, deficiency of nutrients in crops may result in deterioration of final produce quality and hence affect the production level too. Many important nutrients such as nitrogen, phosphorous and magnesium are found to be declining over the time in cereals resulting in to deficiency diseases in people. Since, crops are very sensitive to the weather conditions, in both extreme hot and cold conditions the regular

Table1. List of review articles having coverage on Biosensors in Agriculture

S. No.	Title of articles Analytes detected	Publication resources	Ref
1	Overview on Biosensors for Detection of Organophosphate Pesticides	Curr. Trends Biomedical Eng. Biosci. *5(3)* (2017) : 555663.	1
2	Progressive development in biosensors for detection of dichlorvos pesticide: A review	Environ. Chem. Eng. (2021), 9(2) 105067.	2
3	Biosensors in Agriculture: Recent Trends and Future Perspectives	Springer (2021)	3
4	Fungal disease detection in plants: traditional assays, novel diagnostic techniques and biosensors	Biosens. Bioelectron, 87 (2017), pp. 708-723	4
5	Recent developments in biosensors to combat agricultural challenges and their future prospects	Trends Food Sci Technol, 88 (2019), pp. 157-178	5
6	Smart nanobiosensors in agriculture	Nature Food volume 2, pages 920–921 (2021)	7
7	Implications of Nanobiosensors in Agriculture	Journal of Biomaterials and Nanobiotechnology, 2012, 3, 315-324	8
8	Biosensor for agriculture and food safety: Recent advances and future perspectives	Advanced Agrochem Volume 1, Issue 1, September 2022, Pages 3-6	9
9	Utility Biosensors for applications in Agriculture–A Review	Journal of American Science 2010;6(9)	10

physiochemical processes of plants are disturbed and conditions of stress arises. Technologies for rapid and on-site detection of plant-stress conditions are still the most desirable ones as compared to conventional methods. The current innovations in biosensors, have found applications in agricultural development as well. Biosensors have most significantly contributed to achieve the ever-existing goal of precision in agriculture. [3-6]

The existing current agricultural practices for fulfilling the food requirements include use of natural and artificial inputs which includes indiscriminate use of agrochemicals and sophisticated equipment. These indiscriminate uses of agrochemicals caused significant deterioration of the water, soils and food commodities thereby there is increase levels of pollution in the agricultural environments, which in turn strongly affect the human and animal health and their ecosystem. As per the report, there are 26 million people getting affected due to pesticide poisoning annually on a global basis which in turn causes 220,000 people annual deaths. Some of the pesticides are having nature of persistent organic pollutant (POP) and their residues also persist in natural environment for a prolonged time period. POP also contaminate the soil and ground water and thus raise alarm in the natural ecosystem. In fact, many reports are available which state the presence of pesticide residues in the food chain followed by get accumulated in the body of consumers and causes health issues. Therefore, the monitoring of pesticide residues in the soil, water and agricultural commodities becomes a matter of concern. This will also display the information about the occurrence of agrochemicals is within or beyond the acceptable limits. [1-4]

During the practices of pre-harvest stages, the crop is affected by many natural toxin contamination, diseases, phytohormone imbalance, deficiency of nutrients, irrigation scheduling, lodging and extreme weather conditions (drought and floods). The spread of microbial pathogenic infestation may cause an epidemic form, if not monitored and stopped at proper time. Biosensors can be developed for detection of various microbes, responsible for plant diseases. With the help of biosensors, real-time microbial viability in raw and processed food can be assessed, non-destructively. There are various physiological processes taking place in the plants for producing chemicals in response to abiotic stresses (abnormal temperature, ultraviolet, drought, floods etc.), changes in levels of phytohormones (abscisic acid, gibberellins, cytokinins, auxin, ethylene, salicylic acid etc.), seed dormancy and germination stages. In addition, phytohormones are easily decomposed by external triggers such as heat, light, and oxygen. Thus, the exact level of phytohormones in plants needs to be accurately assessed for providing balanced inputs in agriculture for improving quality of produce, eventually. Post-harvest agriculture is a complex aggregate of operations consisting of harvesting, sorting, storage, processing and packaging. The demand for reliable and inexpensive methods for the assessment of fresh produce quality is expanding. In agriculture the food quality is evaluated based on internal and external quality both. Internal quality itself is determined by various factors such as, nutrients, TSS, pH, acidity, enzymes etc., while external quality is determined by various sensory attributes such as, colour, smell, texture etc. The internal quality attributes though are the most important parameters are still judged by consumers based on sensory examinations only and huge lots are tested using biochemical analysis at laboratory levels. Biosensors offer a viable opportunity to fulfil this niche. Biosensors are now developed for catering very sensitive issues related to raw and processed food. The biosensors have additional advantage of being on-site testing tool for analysis in agriculture. [5-9]

3. Conclusion

This article presents a brief review on scope of biosensors in the field of agriculture. Biosensors have many applications at different stages of crop plants production starting from soil conditions to the post-harvest and consumption of the agricultural products. It has listed the gist of the biosensor's implications in agriculture. Biosensors will have additional advantage of being on-site monitoring system for analysis in agriculture.

4. Acknowledgement:

Authors are grateful to their institute for providing financial support and facilities.

References:

1. J. Kumar, J.S. Melo, *Curr. Trends Biomedical Eng. Biosci., 5(3)* (**2017**): 555663. DOI: 10.19080/CTBEB.2017.05.555663.
2. A. Mishra, J. Kumar, J. S. Melo, Bhanu Prakash S, *J. Environ. Chem. Eng.* (2) **(2021)** 105067.
3. R.N. Pudake, U. Jain, C. Kole, Biosensors in Agriculture: Recent Trends and Future Perspectives, Springer **(2021)**.
4. M. Ray, A. Ray, S. Dash, et al. *Biosens. Bioelectron*, 87 (**2017**) 708-723.
5. M. Kundu, P. Krishnan, R.K. Kotnala, G. Sumana, Trends Food Sci Technol, 88 (**2019**) 157-178.
6. Arben Merkoçi, Nature Food, 2 **(2021)** 920–921.
7. V. Rai, S. Acharya, N. Dey, Journal of Biomaterials and Nanobiotechnology, 3 **(2012)** 315-324.
8. X. Wang, Y. Luo, K. Huang, N. Cheng, Advanced Agrochem, 1**(2022)**3-6.
9. J.S Rana, J. Jindal, V. Beniwal, V. Chhokar, Journal of American Science, 6 **(2010)**.

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Biosensor for healthcare application: An overview of electrochemical biosensors for metabolites and cancer biomarker

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Abstract

Biosensor is an analytical device wherein the analyte quantification is enabled by a bioreceptor coupled transducer system which converts the biological response into a detectable signal. Biosensors are gaining remarkable attention in the healthcare sector due to their high potential for application in clinical diagnosis and point-of-care testing. Recent advances in materials chemistry has led to a plethora of functional materials which have significantly influenced the biosensor device to give accurate, rapid, cost-effective diagnosis for variety of diseases. Because of the increasing rate of cancer incidence, large attention has been focused on biosensing systems for cancer diagnosis. Accurate detection of cancer biomarker facilitates early diagnosis and increases the chance of survival of cancer patients. Additionally, biosensors for physiologically important metabolites also play a key role in healthcare sector. The biosensors for healthcare are being developed in different formats like wearable, flexible, paper based, microfluidics, self-powered etc. This article presents an overview of recent development in the area of biosensor for detection of important metabolites and cancer biomarkers. Contribution of our group towards the development of conducting polymer and nanomaterials based biosensors for detection of metabolites (glucose, uric acid, H_2O_2) and cancer biomarkers (alphafetoprotein, carcinoembryonic antigen etc.) is also presented.

Introduction:

The biosensor is an analytical device that estimates the analyte using integrated bioreceptor-transducer system which converts the biological response to the analyte into a detectable signal. Biosensors for detection of disease-related biomarkers or metabolites are important in the area of biomedical research, clinical diagnosis and point-of-care testing. Detection of these targets shows great promise for early detection of disease and also helps to understand the biological processes and response associated with disease progression [1].

Cancer is the second leading cause of death worldwide after heart diseases [2]. Detection of cancer biomarker (CB) has great clinical importance in screening and early diagnosis of cancer [3]. Detection of metabolites (like glucose, uric acid, lactate, creatinine etc) is another area of importance in medical diagnostics. Proper monitoring of glucose can decrease the risks of several diabetes-induced diseases, allowing the patients to continue a healthy lifestyle. Self-testing blood glucose monitors currently being used are essentially electrochemical sensors which flourished between 1980s to 1990s. Last two decades has seen a lot of development in biosensor designed in different format like paper based, implantable, wearable, self-powered, tattoo based, flexible, flow-through sweat sensor, microfluidics, skin-like sensors especially for healthcare purpose [4]. These varied formats pave the way for rapid, safer and cost-effective diagnosis.

After Clark's invention of electrochemical biosensor for glucose detection, several kinds of biosensor for different analytes have been developed for various diseases. Since then, biosensors have been extensively investigated for detection of metabolites, cancer biomarkers, nucleic acids, viruses and cells. Different techniques like fluorescent, electro-chemiluminescent, colorimetry, enzyme-linked immunosorption assay, electrochemistry and surface plasmon resonance [5] are utilized for biosensing purpose. In comparison to aforementioned methods, electrochemical technique offer several advantages such as portability, ease of miniaturization and low fabrication cost. In an electrochemical biosensor, electrode material (also known as immobilization matrix or sensing platform) and sensor design play an important role in the performance of the device [4]. The electrode material used for the biosensor construction is known as transducer which converts biological signal to detectable signal. Ideal electrode material for biosensing system enables efficient immobilization of biomolecules without loss of its bioactivity and facilitates electron transfer from biomolecules to electrode. For instance, polyaniline (PANI), a conducting polymer, facilitates the covalent binding of enzymes or other bioreceptor by simple glutaraldehyde coupling reaction and enables hassle-free electron transfer. However, in some bioreceptor, electron transfer is not easy due to the protein insulation of active site (e.g. horseradish peroxidase, HRP). In such cases, redox mediators like hydroquinone are used to shuttle the electron between enzymes and electrode.

Generally, carbon, metal nanoparticles, metal oxides, inorganic complexes, conducting polymers and biopolymers based materials are being used as the transducer for biosensor. Immobilization of bioreceptor on transducer plays a key role in the performance of biosensors. For example, linear range, sensitivity and stability of biosensor depend on the strategy used for immobilization of bioreceptor on transducer. Three general methods of immobilization are physical adsorption, entrapment and covalent binding. Among these strategies, covalent binding leads to increased stability of sensing platform allowing the bioreceptor improving the shelf-life of the biosensor [5, 6]. In this article, recent developments in electrode materials and sensor device fabrication for metabolites and cancer biomarker detection have been summarized.

Electrochemical biosensor:

Among various detection methods, electrochemical method has received increasing attention because of simple instrumentation, portability, high sensitivity and rapid analysis. In the electrochemical biosensor, signal is generated by the interaction/binding between the target species and bioreceptor on the transducer. This signal could be a change the output current, number of electrons exchanged through an oxidation-reduction or charge transfer resistance resulting in amperometry, voltammetry and electrochemical impedance spectroscopy (EIS) based sensing system, respectively. Amperometry is one of the widely used technique which measures current resulting from the oxidation or reduction of an electroactive species in a biochemical reaction occurring at a constant applied potential. In voltammetry based biosensor, potential of target species bound transducer is ramped from lower to higher voltage with respect to reference electrode and the resulting current associated with the redox reaction is measured. Different voltammetry techniques like cyclic (CV), linear sweep (LSV), differential pulse (DPV), square wave (SWV) and stripping (SV) voltammetry are routinely used for biosensing purpose. Among these, DPV and SWV are most commonly used for the cancer biomarker detection. Electrochemical impedance spectroscopy (EIS) is a sensitive tool for the analysis of biomolecular interactions occurring at the modified transducer surface. Impedimetric biosensors evaluate the changes in charge transfer resistance and capacitance at surface of transducer during the selective binding of the target. In EIS based biosensor, during binding of antigen to antibody bound electrode, significant increase in the charge transfer resistance is generally observed. In impedometric immunosensors, conducting polymers are generally used to immobilize the antigens, commonly through covalent attachment, thus allowing the detection of the antibodies due to the high antigen-antibody affinity [6].

Biosensors for metabolite detection:

Metabolic diseases are chronic diseases resulting from metabolic disorders which lead to the change in concentration of metabolites like sugar, fat, electrolyte, proteins and purine in physiological fluids like blood serum or urine [7]. Deviation in the concentration of metabolites from the normal range indicates the existence of disorders and harms the human body. Hence, detection of metabolites in physiological fluids is very important for effective treatment. Diabetes mellitus is one of the most common metabolic diseases caused due to insulin deficiency and may lead to elevated blood glucose levels. Determination of glucose in sweat is being extensively attempted since it is non-invasive and easy to collect as compared to blood [8].

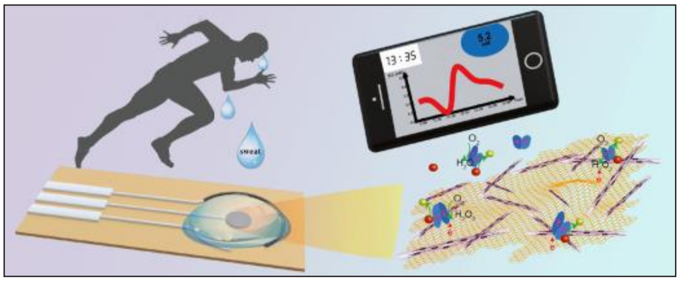

Fig 1: Silk-Based Electrochemical Sensor for the Detection of Glucose in Sweat (Reproduced with permission from ref. 8. Copyright (2022) American Chemical Society)

Lactate level in body fluid is an important health indicator since it is related to several ailments, such as pressure ischemia, cystic fibrosis, metabolic disorders, hypoxia, heart failure and renal failure. Determination of lactate level in blood and sweat is important to assess the exercise level of the sports person under anaerobic metabolism conditions to prevent metabolic acidosis and prevent body damage by severe work out. Blood and sweat lactate level in healthy people at rest condition are about 0.5- 1.5 mM and 5-25 Mm, respectively. Therefore, it can offer enormous benefits if lactate level can be monitored in real-time and in a non-invasive manner. Schematic representation for an on-body and non-invasive sweat analysis for lactate detection is shown in **Fig 2** [9].

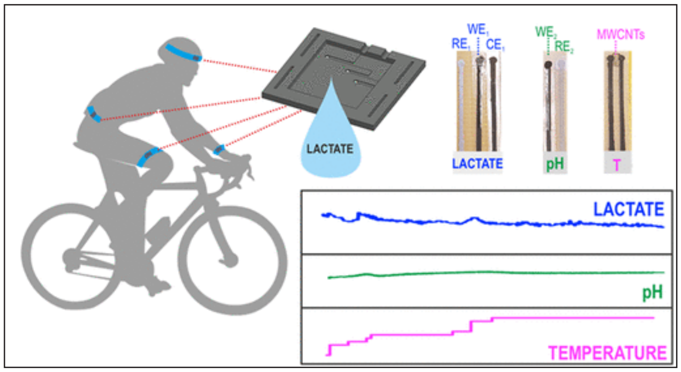

Fig 2: On-body and non-invasive sweat analysis for lactate detection (Reproduced with permission from ref. 9. Copyright (2021) American Chemical Society)

Uric acid is a metabolite presents in urine or serum excreted by the kidney and is the end-product of purine nucleotide metabolism. The elevated concentration of uric acid in physiological fluids is associated with hyperuricemia, kidney disease, gout and cardiovascular diseases. Guo demonstrated the world's first medical smart-phone as an electrochemical analyzer, which is integrated with an enzymatic investigation strip for detection of uric acid in blood (**Fig 3**) [10].

Fig 3: Uric acid monitoring with a smartphone as the electrochemical analyzer (Reproduced with permission from ref. 10. Copyright (2016) American Chemical Society)

Ceratinine is a metabolite which exists in urine and blood and its amount helps the physicians to assess the status of kidneys. Elevation of creatinine level in physiological fluids is associated with diseases like renal, muscular and thyroid functions. Recently Singh et al., fabricated an electrochemical chip modified with reduced graphene oxide stabilized binary copper–iron oxide-based nanocomposite (**Fig 4**) for detection of creatinine in blood. The biosensor had a wide linear range (0.01-1000 µM) and low detection limit of 10 nM [11]. Hence, detection of glucose, lactate, uric acid and ceratinine are imperative in healthcare sector.

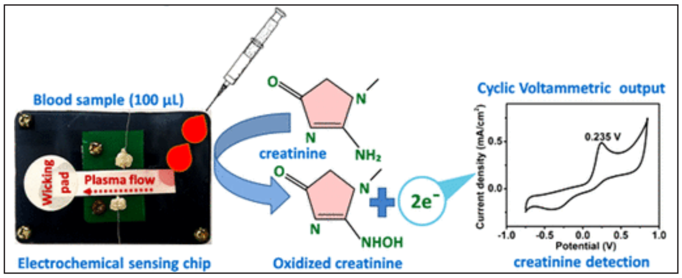

Fig 4: Schematic diagram of reduced graphene oxide based creatinine detection (Reproduced with permission from ref. 11. Copyright (2021) American Chemical Society)

Development in fabrication methods for metabolite biosensor:

Development in fabrication platform combines electrochemical methods with advance device technologies to offer different types of efficient capabilities in sensing device. Most of these systems employ flexible circuit systems which can be used in wearable or implantable devices. Paper based microfluidics device was reported first during 2009 for simultaneous detection of multiple metabolites (glucose, lactate and uric acid). The biosensor was fabricated using photolithography and screen printing technology (**Fig 5**) [12]. This invention used simple chemical modification, disposable electrodes and low electric powered simple instrumentation for cost-effective on-site or point-of-care application. Following this invention, implantable sensor for long-term monitoring of glucose in tissue by wireless telemetry has been proposed. This sensor operated continuously for more than a year when

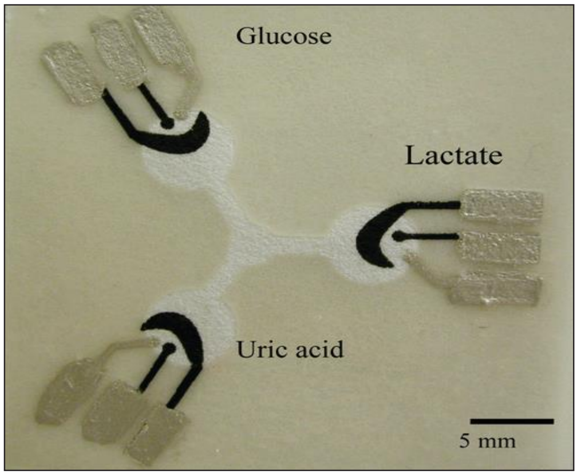

Fig 5: Picture of three electrode paper-based microfluidic devices. The hydrophilic area at the center of the device wicks sample into the three separate test zones where independent enzyme reactions occur. The silver electrodes and contact pads are made from Ag/AgCl paste with the black electrode portions being the PB-modified carbon electrodes. The device size is 4 cm × 4 cm. (Reproduced with permission from ref. 12. Copyright (2009) American Chemical Society)

implanted in subcutaneous tissues of pig having diabetes demonstrating its potential for human applications [13]. Liao et al presented wireless, non-invasive and a fully integrated active contact lens system for continuous real-time monitoring of glucose levels. The sensor interface used the tear fluid for metabolite detection which reduced the sampling procedures and potential of infection during sample collection [14].

In recent years, development of wearable biosensors for metabolites has attracted remarkable interest for next-generation, non-invasive health monitoring systems. However, size, weight and powering of such device are more critical parameters during device fabrication. Such devices can be powered by the use of enzymatic biofuel cells which harvest energy from the metabolites present in body-fluids. Major advances over the last decade has led to the development of stretchable, textile-based, self powered sensors using sweat or blood metabolites as the fuel. Despite exponential development of self-powered biosensors, most of them are still in the proof-of-concept stage.

Integrating sensing electrodes on flexible polymer matrix enables wearable systems that can bend, stretch and adhere to body without sacrificing sensing performance comparative to conventional sensing platforms. The mechanical flexibility allows the system to wrap around the wrist, thereby providing a direct sensing of lactate in sweat for on-body measurements. Wearable and flexible biosensor using molecularly imprinted Ag nanowires and PEDOT modified screen printed electrode has been fabricated for noninvasive monitoring lactate in human sweat [15, 16]. Hossain and Slaughter proposed flexible and fully integrated biosensor for glucose and uric acid using pyrenebutanoic acid functionalized laser scribed graphene [17]. Non-invasive, flexible ceratinine nano-sensor has been developed based on triboelectric nanogenerator using PANI based materials. Operation of sensor over a large range of bending angles enabled the application of wearable devices for personalized health monitoring (Sensors and Actuators A: Physical 320 (2021) 112585) [18]. Ultra-small and flexible biosensor for sweat analysis is shown in **Fig 6** [19].

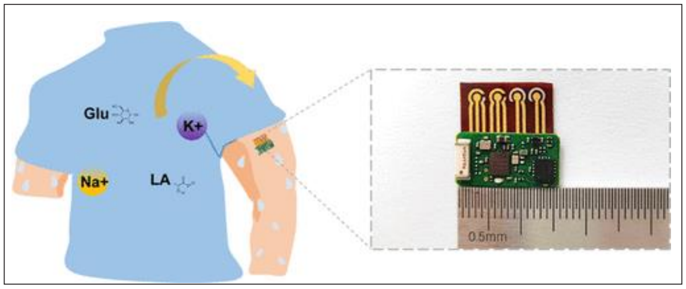

Fig 6: Wearable biosensor for sweat analysis (Reproduced with permission from ref. 19. Copyright (2022) American Chemical Society)

Most of the flexible and wearable electrochemical sensor devices focus on the detection of metabolites from the physiological fluids like sweat, tear fluid and serum. However, larger biomarkers like proteins and bio-macromolecules are comparatively less explored which could be due to the challenges in immobilization of antibodies and signal transduction on flexible substrate. Moreover, fabrication of flexible sensing platform utilizes the insulating polymer which limits the electron transfer between the enzyme and electrode. Xu and Yadavalli demonstrated label-free impedimetric biosensor device constructed by photolithography on flexible and free-standing fibroin substrates for detection of vascular endothelial growth factor (VEGF) in human blood serum and on tissue surface. Proposed sensor was biocompatible and can be used for the early diagnosis of cancer (**Fig 7**) [20].

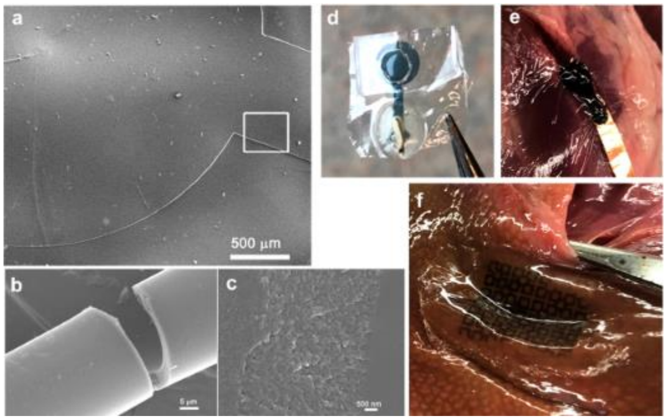

Fig 7: SEM images of (a) flexible sensor electrode, (b) flexible sensor electrode in a rolled-up position, (c) close-up of the electrode surface marked by white rectangle (d) free-standing flexible biosensor electrode that can be conformable to soft tissue, (e) and (f) in situ detection of biomarkers at tissue interfaces (Reproduced with permission from ref. 20. Copyright (2019) American Chemical Society)

The development of electrochemical microfluidic POC testing platform affords rapid analysis with portability, which requires low volumes of sample. Uliana et al., constructed a disposable electrochemical microfluidic device for sensitive and rapid detection of a cancer biomarker estrogen receptor alpha in MCF-7 cell lysates. This sensor was portable, flexible, scalable, and employed low cost material for electrode fabrication [21].

Fabrication of electrodes by screen-printing is an important substitute to photolithography technique which can offer inexpensive, scalable and disposable sensors. Moreover, screen printed electronics have gained attention due to its simple designing protocol and ability to fabricate

3D structures. For example, carbon, conducting polymer and gold nanoparticle based inks have been developed which can be used for mass production of electrode arrays for immunosensing. Immunoassays based on screen printed electrode on paper based matrix coupled with microfluidic system have been used to detect panel of cancer biomarkers. Wang et al., reported label-free microfluidic paper-based electrochemical aptasensor for ultrasensitive and simultaneous detection of carcinoembryonic antigen and neuron-specific enolase in a clinical sample. The paper-based sensing device was fabricated through wax printing and screen-printing, which enabled functions of sample filtration and sample auto injection [22].

Wu et al fabricated paper-based microfluidic electrochemical device integrated with amplification-by-polymerization for the detection of multiple cancer biomarkers. Polymer formed in the detection probe during binding event provides numerous active sites for HRP which amplifies the electrochemical signal. Graphene present in the sensing platform accelerated electron transfer and thus amplified the signal [23]. Tang et al demonstrated injet-printed gold compact disc based cost-effective electrochemical immunosensor device for the sensitive and rapid detection of multiple cancer biomarkers. This method afforded wide linear range from 10 to 1300 fg/ml for cancer biomarker interleukin-6 concentration in diluted human blood serum with detection limit of 10 fg/ml, which is 200 times lower than HRP mediated conventional 3 electrode system based immunosensors [24].

Materials for transducer and immobilization matrix:

Electrochemistry based biosensing methods provide simple and miniaturized analytical devices for point-of-care analysis which can give complementary test reports to the conventional imaging techniques. Developing such sensing device needs robust materials which could provide high sensitivity, selectivity, reproducibility and stability during detection events. In any electrochemical biosensor, the role of sensing platform or immobilization matrix is very important. Immobilization matrix binds enzyme or antibody which can specifically interacts with target species and then convert the biological response to electrochemical signal. For that, the matrix needs to have dense functional group and conductivity to immobilize enzymes or antibody and transfer electrons respectively. Functional nanomaterials endow the large surface area, necessary functional groups and reasonable conductivity which facilitate efficient immobilization of enzymes or antibodies and easy electron transfer between enzymes to electrode. The functional materials employed as electrodes (also known as sensing platform or immobilization matrix) should facilitate electro-catalytic reaction, electron movement and should be biocompatibility. Several materials based on carbon, conducting polymers, biopolymers, metal nanoparticles, metal oxides, inorganic complexes, metal organic framework have been widely used in the sensing platform. The enormous development in the area of functional materials over past decades has made a huge impact on the performance of biosensors.

Carbon based materials like carbon nanotubes, graphene are extensively utilized material for biosensors due to their structural features, electronic conductivity and easy functionalization. Aligned CNT arrays with GOx immobilized on edge of CNT could connect the enzyme and electrode by acting as molecular wires enabling direct electron transfer [25, 26]. Reduced graphene oxide was covalently modified with redox molecule and L-lactate dehydrogenase for lactate detection in human blood serum. The covalently grafted redox molecule efficiently mediated the oxidation of NADH at 40 mV which is significantly less than that for unmodified electrode. Graphene not only facilitated functionalization and enzyme immobilization but also facilitated the electron transfer between active site of enzyme to electrode [27]. Recently, ultra-small iron oxide decorated nitrogen doped carbon was used to detect uric acid in human urine with high sensitivity and selectivity. Sensor afforded wide linear range from 2–200 µM with low detection limit (0.29 µM) for uric acid detection without any interference from other bio-analytes [28]. Two enzymes lactate oxidase and HRP were encapsulated in metal organic framework and used for intracellular lactate detection [29].

Our group integrated PANI with biopolymer pectin (PEC) to detect metabolite glucose [30]. PANI NPs were synthesized by our group using biopolymer pectin a stabilizer and functionalized with GOx to fabricate a highly specific and sensitive biosensor for glucose detection (**Fig 8**). Pectin not only stabilized the colloidal PANI NPs but also decreased the particle size, increased the active functional groups and surface area of PANI nanoparticles and thus facilitated the enhanced immobilization of GOx via covalent coupling. Moreover, PEC arrested enzyme leaching and improved stability of biosensor to afford high sensitivity, wide linear and a low detection limit at a working potential of 0.6 V (RSC Adv., 2014, 4, 40917) [30]. Furthermore, pectin stabilized PANI NPs were decorated with Ag NPs (Ag@PANI-PEC) and used as the electrode material for physiological fluids operatable supercapacitor. Due to the use of biopolymer PEC, Ag@PANI-PEC exhibited biocompatibility and the presence of silver on Ag@PANI-PEC rendered antibacterial properties making composite an ideal material for in vivo implants. These findings established the feasibility of using the Ag@PANI-PEC as a potential material for energy storage device in self-powered supercapacitor [31]. In another work, mesoporous PANI was synthesized using mixture of surfactants and used as the immobilization matrix for glucose and H_2O_2 sensing (**Fig 9**). PANI thus synthesized exhibited H3 hysteresis loop, which shows that materials have non-rigid aggregates like particles and signify the presence of slit shape pores. The resulting PANI exhibited significantly higher surface area and pore volume than conventional PANI [32].

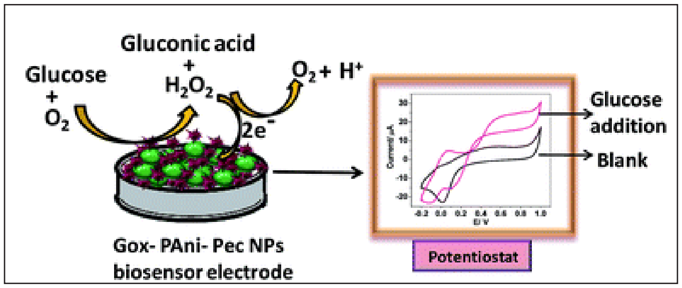

Fig 8: PANI-PEC based biosensor for glucose detection [30]

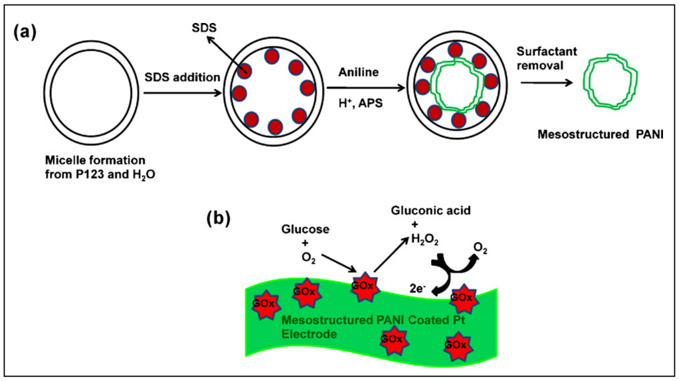

Fig 9: Schematic representation of the proposed mechanism for the formation of mesostructured PANI by the self-assembly of SDS and tri-block copolymer. (b) Modification of PANI-SDS-P123/PANI electrodes for efficient detection of glucose. (Reproduced with permission from ref. 32. Copyright (2012) Elsevier)

PB is an important material extensively used for sensing application due to its electro-catalytic property especially for H_2O_2 reduction. Our group synthesized PANI-PB composites by different synthesis protocols and studied its energy storage and H_2O_2 sensing property. The spatial distribution of PB with respect to PANI, particle size and PB content was found to affect the sensing capability of the resulting composite prepared by different synthesis approach. It was observed that PANI coated PB material provided excellent energy storage property whereas PB coated and embedded PANI provided excellent H_2O_2 sensing property [33]. In another work, PANI-PB composite was electrochemically synthesized on Pt electrode and covalently modified with enzyme uricase to study the biosensor for detection of uric acid. Sensor operated at low working potential (0 V vs Ag/AgCl) and afforded high sensitivity and wide linear range for uric acid detection (**Fig 10**) [34].

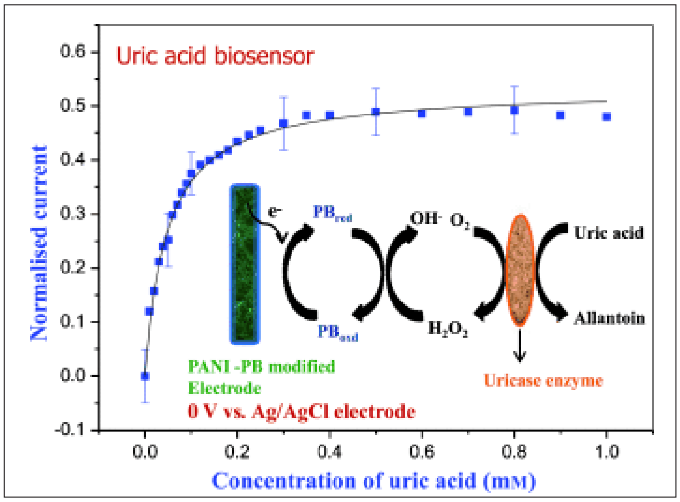

Fig 10: PANI-PB based biosensor for uric acid detection (Reproduced with permission from ref. 34. Copyright (2013) Wiley)

Biosensors for detection of cancer biomarkers

For cancer biomarker detection numerous combination of materials are being explored to achieve sensitive and accurate detection [35-37]. Barhoum and Forster developed simple strategy to detect cervical cancer biomarker MCM5 on thiol covered gold electrode using $[Fe(CN)_6]^{3-/4-}$ redox probe [35]. Venkatanarayanan et al demonstrated label-free detection of ovarian cancer cells, SKOV3, onto platinum microelectrodes modified anti-EPCAM capture antibodies with high sensitivity using electrochemical impedance spectroscopy [38].

Most of the sandwich electrochemical biosensors use enzyme [39] or metal ions [40] or redox molecules [41] conjugated antibody to bind with antigen bound electrode to impart or enhance the signal. In contrast with passivation approach, the sandwich assay provides an increase in electrochemical signal as the concentration of target increases. Label used for conjugating and carrier used for loading those labels play a key role in signal enhancement. Labels like HRP enzyme conjugated to antibody enhances the response after binding with immune-complex bound electrode in presence of H_2O_2 and redox mediator. Loading this HRP conjugated antibody on metal [39] or carbon based [42] carriers further enhances the electrochemical signal to afford ultrasensitive detection system. Conjugation of different metal ions [40] and redox molecules [41] with different types of antibody has another advantage of detecting multiple biomarkers simultaneously in single sample which will facilitate accurate diagnosis of various ailments.

Our lab is extensively involved in development of biosensor for cancer detection using different strategies discussed above. In one of our earlier work, PANI was modified with biopolymer bovine serum albumin and Ag NPs and used as the enriched immobilization matrix for the detection of α-fetoprotein (AFP), a liver cancer biomarker, in human blood serum. The matrix entrapped large amount of capture antibody due to its higher surface area and amine-rich nature. The detection probe consists of HRP-AFP antibody loaded Ag NPs. The detection probe enabled immobilization of higher amount of enzyme HRP on its surface leading to enhanced electrochemical signal during its binding with AFP. In the sandwich biosensor, signal amplification was bestowed by enriched immobilization matrix as well as the detection probe. The biosensor demonstrated dual amplification due to immobilization matrix and detection probe bio-functionalized Ag NPs containing HRP conjugated AFP antibody. Sensor displayed linear range from 0.01 to 10 ng/ml with detection limit 4.7 pg/ml. This dual amplification strategy has potential for application in detection of other cancer biomarkers in clinical diagnosis (**Fig 11**) [43].

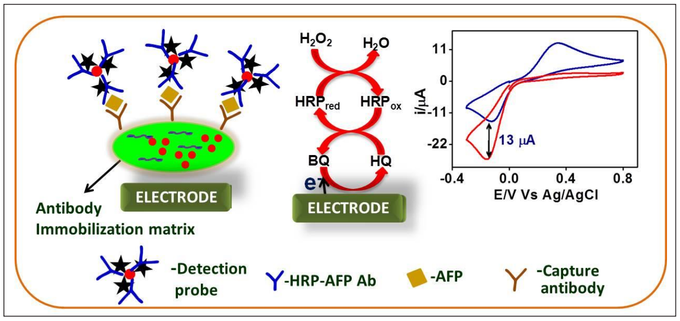

Fig 11: Schematic representation of PANI-BSA-Ag NPs based biosensor for AFP detection (Reproduced with permission from ref. 43. Copyright (2020) Wiley)

In another work, an organically modified PANI containing large number of carboxylic acid groups (carboxylic acid tethered PANI, PCOOH) was synthesized using thiol-ene "Click Chemistry". Cyclic voltammetry studies on PCOOH was carried out in physiological fluids like PBS, blood, serum, urine and sweat and culture media like DMEM and LB broth. Cyclic voltammogram (CV) of PCOOH displayed redox transition at 0.1 V vs Ag/AgCl even at neutral pH due to leucomeraldine to pernigraniline conversion facilitated by the proton from –COOH group of covalently grafted mercaptoundecanoinc acid on PANI chain. However, CV of unmodified PANI did not display redox peak in above mentioned physiological fluids and culture media. This organically modified PANI poses advantage in terms of operational stability vis-`a-vis doping with polymeric acids which could leach out during continuous operation in neutral media. The specific capacitance of PCOOH in PBS, blood, serum, urine, sweat, DMEM, LB broth at 1 A/g was found to be 215, 305, 105, 234, 190, 126, 182 F/g respectively, which is significantly higher than that of conventional PANI in corresponding physiological fluids and culture media (8, 1, 1.5, 9, 75, 2, 1 F/g respectively). Furthermore, PCOOH displayed biocompatibility towards L929 cells which was analyzed by MTT assay, where it displayed cell viability of 92.11 ± 5.84 %. Also, PCOOH exhibited good cyto-compatibility during continuous voltage cycling in A549 cell lines where 96.8 % cell viability was retained even after 1000 galvanostatic charge–discharge cycles (**Fig 12**) [44].

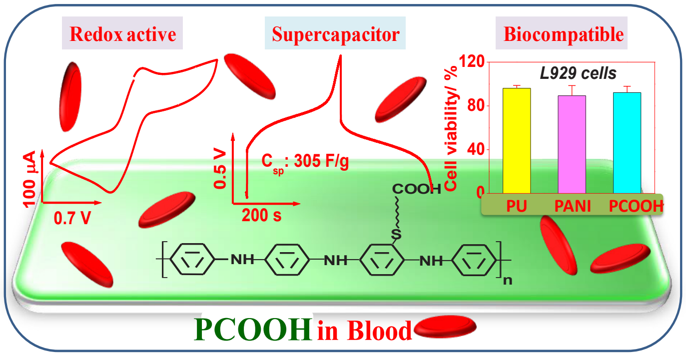

Fig 12: Energy storage property of PCOOH in blood (Reproduced with permission from ref. 44. Copyright (2022) Elsevier)

Further, our group has demonstrated use of PCOOH as an efficient immobilization matrix in biosensor for detection of metabolites (glucose, uric acid and H_2O_2) and cancer biomarker (AFP). Immobilization efficiency of PCOOH was investigated by surface plasmon resonance and fluorescence microscopic analysis which revealed dense immobilization of biomolecules on PCOOH as compared to conventional PANI. Inherent conductivity and high immobilization efficiency of PCOOH helped to avoid the use of other nanomaterials in the immobilization matrix. A sandwich electrochemical biosensor constructed using PCOOH for detection of AFP displayed excellent sensitivity with good specificity, reproducibility, wide linear range (0.25–40 ng/ml) and a low detection limit of 2 pg/ml. The biosensor was validated by estimating AFP in human blood serum samples where the AFP concentrations obtained are consistent with the values estimated using ELISA. Enzymatic biosensor was constructed by covalent immobilization of GOx, uricase, and HRP on PCOOH for

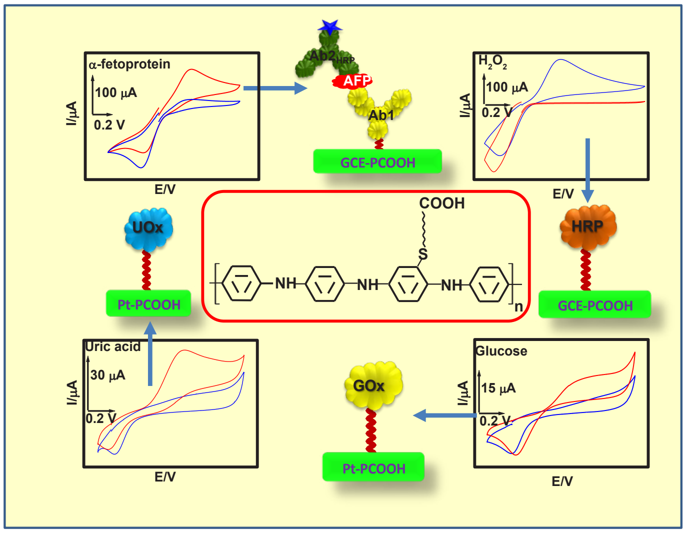

Fig 13: Schematic representation of carboxylic acid tethered PANI based generic bioassay for metabolites and cancer biomarker detection (Reproduced with permission from ref. 45. Copyright (2021) Springer Nature)

detection of glucose, uric acid, and H_2O_2, respectively. Sensors displayed reasonable sensitivity and linear ranges with a detection limit of 10, 1, and 8 μM for glucose, uric acid, and H_2O_2, respectively. Thus, PCOOH offers a great promise as a sensing platform for development of high-performance biosensors to quantify a variety of other disease biomarkers (**Fig 13**) [45]. Recently, a sandwich electrochemical immunoassay has been developed in our lab for simultaneous detection of two cancer biomarkers alphafetoprotein (AFP) and carcinoembryonic antigen (CEA) using redox molecule grafted bio-functionalized nanoparticles.

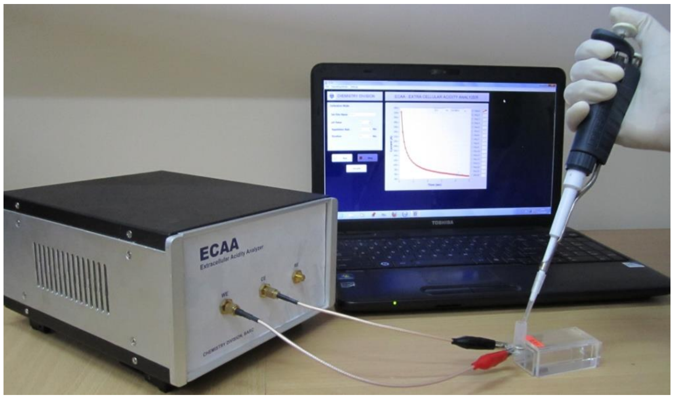

Fig 14: ECAA setup developed at Chemistry Division, BARC

Cancer cells are known to have high rate of glycolysis due to abnormal metabolism and rapid growth. We have developed a portable electrochemical device, namely Extra-Cellular Acidity Analyzer (ECAA), which is designed to sense the difference in rate of glycolysis between cancer and normal cells/tissues. (Figure 14) Thus, it can be used as an indirect tool to detect cancer. ECAA enables direct measurements using tissue from biopsy samples for point-of-care as well as pathology lab based application. ECAA technology has been transferred to four private companies for commercialization. (ECAA Technology code MD12ChD; http://barc.gov.in/technologies/ecaa/index.html)

Conclusion:

This article presents an overview of the development in materials and device fabrication methods for electrochemical biosensor focusing on metabolites and cancer biomarker detection. Carbon, metal nanoparticles, conducting polymer and biopolymer based materials are extensively utilized for construction of electrochemical biosensors. These materials or their composites not only improve the immobilization efficiency of the matrix but also acts as transducer to convert the biological signal to electrochemical output. As far as device fabrication is concerned, recent years have witnessed tremendous advancements in biosensors for metabolites detection in various formats like wearable, flexible and paper based devices for on-body measurements. For continuous monitoring, wrist watch type and contact eye lens based devices have been demonstrated. These devices utilized photolithography, screen printing and inkjet printing for electrode fabrication on flexible substrates which gave excellent performance even during motion. Among these electrode fabrication techniques, screen printing method is cost-effective and used mainly for carbon and conducting polymer based electrode materials. The article also describes examples of nanomaterials which are used to improve the sensitivity, specificity, stability, linear range of the biosensor. For cancer biomarker detection, even though a lot of development is being witnessed in synthesis of biosensor materials, developments in device fabrication are still in proof-of-concept stage. This could be due to number of bio-functionalization steps involved and stability of bioreceptors during sensor device fabrication. However, it is essential to fabricate portable and point of care testing device using robust material for sensitive, accurate and rapid detection of biomarkers in body fluid to cut down the cancer related mortality. Contribution of our group towards the development of polyaniline based materials using surfactants, biopolymer, metal nanoparticles, inorganic complex towards sensing of metabolites (glucose, uric acid and H_2O_2) and cancer biomarker has been discussed. The development in materials and device fabricating technologies has led to biosensors with potential to detect and quantify metabolites and cancer biomarkers in point of care testing and on-body monitoring systems. Thus, electrochemical biosensors have immense potential to contribute in the healthcare sector for disease diagnosis.

References:

1. L. Farzin, M. Shamsipur, L. Samandari, S. Sheibani, *Microchim. Acta* **2018**, *185*, 276.
2. N.G. Zaorsky, T.M. Churilla, B.L. Egleston, S.G. Fisher, J.A. Ridge, E.M. Horwitz, J.E. Meyer, *Ann. Oncol.*, **2017**, *28*, 400.
3. J. Wu, Z.F. Fu, F. Yan, H.X. Ju, *Trends Anal. Chem.*, **2007**, 26, 679.
4. H. Teymourian, A. Barfidokht, J. Wang, *Chem. Soc. Rev.*, **2020**, *49*, 7671.
5. L. wu, X. Qu, *Chem. Soc. Rev.*, **2015**, *44*, 2963.
6. N. Wongkaew, M. Simsek, C. Griesche, A. J. Baeumner, *Chem. Rev.*, **2019**, *119*, 120.
7. A. Koklu, D. Ohayon, S. Wustoni, V. Druet, A. Saleh, S. Inal, *Chem. Rev.*, **2022**, *122*, 4581.
8. Q. Chen, Y. Liu, K. Gu, J. Yao, Z. Shao, X, Chen, *Biomacromolecules* **2022**, *23*, 3928.
9. X. Xuan, C. Pérez-Ràfols, C. Chen, M. Cuartero, G. A. Crespo, *ACS Sens.*, **2021**, *6*, 2763.
10. J. Guo, *Anal. Chem.*, **2016**, *88*, 11986.
11. P. Singh, S. Mandal, D. Roy, N. Chanda, *ACS Biomater. Sci. Eng.*, **2021**, *7*, 3446.
12. W. Dungchai, O. Chailapakul, C. S. Henry, *Anal. Chem.*, **2009**, *81*, 5821.
13. D. A Gough, L. S Kumosa, T. L Routh, J. T Lin, J. Y. Lucisano, *Sci. Transl. Med.*, **2010**, *2*, 42ra53.
14. Y. T. Liao, H. Yao, A. Lingley, B. Parviz, B. P. Otis, *IEEE J. Solid-State Circuits* **2012**, *47*, 335.
15. Q. Zhang, D. Jiang, C. Xu, Y. Ge, X. Liu, Q. Wei, L. Huang, X. Ren, C. Wang, Y. Wang, *Sens. Actuators B Chem.*, **2020**, *320*, 128325.
16. C. Zhu, Y. Xu, Q. Chen, H. Zhao, B. Gao, T. Zhang, *J. Colloid Interface Sci.* **2022**, *617*, 454.
17. M. F. Hossain, G. Slaughter, *Bioelectrochemistry* **2021**, *141*, 107870.
18. Y. Luo, T. Zhao, Y. Dai, Q. Li, H. Fu, *Sens. Actuators A: Phys.*, **2021**, *320*, 112585.
19. J. Wang, L. Wang, G. Li, D. Yan, C. Liu, T. Xu, X. Zhang, *ACS Sens.*, **2022**, *7*, 3102.
20. M. Xu, V. K. Yadavalli, *ACS Sens.*, **2019**, *4*, 1040.
21. C. V. Ulianaa, C. R. Peveraria, A. S. Afonsob, M. R. Cominettic, R. C. Faria, *Biosens. Bioelectron.*, **2018**, *99*, 156.
22. Y. Wang, J. Luo, J. Liu, S. Sun, Y. Xiong, Y. Ma, S. Yan, Y. Yang, H. Yin, X. Cai, *Biosens. Bioelectron.*, **2019**, *136*, 84.
23. Y. Wu, P. Xue, K. M. Hui, Y. Kang, *Biosens. Bioelectron.*, **2014**, *52*, 180.
24. C. K. Tang, A. Vaze, J. F. Rusling, *Lab Chip* **2012**, *12*, 281.
25. F. Patolsky, Y. Weizmann, I. Willner, *Angew Chem Int Ed Engl.* **2004**, *43*, 2113.
26. J. Liu, A. Chou, W. Rahmat, M. N. Paddon-Row, J. Justin Gooding, *Electroanalysis* **2005**, *17*, 38.
27. B. Manna, C. Retna Raj, *J. Mater. Chem. B* **2016**, *4*, 4585.
28. T. Tang, M. Zhou, J. Lv, H. Cheng, H. Wang, D. Qin, G. Hu, X. Liu, *Colloids Surf B Biointerfaces* **2022**, *216*, 112538.
29. Y. Zhang, L. Xu, J. Ge, *Nano Lett.*, **2022**, *22*, 5029.
30. B. Thakur, C. A. Amarnath, S. N. Sawant, *RSC Adv.*, **2014**, *4*, 40917.
31. C.A. Amarnath, N. Venkatesan, M. Doble, S.N. Sawant, *J. Mater. Chem. B* **2014**, *2*, 5012.
32. M.U. Anu Prathap, B. Thakur, S. N. Sawant, R. Srivastava, *Colloids Surf B Biointerfaces* **2012**, *89*, 108.
33. C. A. Amarnath, S. N. Sawant, *Electrochim. Acta* **2019**, *295*, 294.
34. B. Thakur, S. N. Sawant, *ChemPlusChem* **2013**, *78*, 166.
35. A. Barhoum, R. J Forster, *Anal. Chim. Acta* **2022**, *1225*, 34022.
36. H. Chen, B. Zhang, Y. Cui, B. Liu, G. Chen, D. Tang, *Anal. Methods* **2011**, *3*, 1615.
37. D. O. Lopez-Cantu, R. B. González-González, E. M. Melchor-Martínez, S. A. H. Martínez, R. G. Araújo, L. Parra-Arroyo, J. E. Sosa-Hernández, R. Parra-Saldívar, H. M. N. Iqbal, *Int. J. Biol. Macromol.*, **2022**, *194*, 676.
38. A. Venkatanarayanan, T. E. Keyes, R. J. Forster, *Anal. Chem.*, **2013**, *85*, 2216.
39. J. Guo, X. Han, J. Wang, J. Zhao, Z. Guo, Y. Zhang, *Anal. Biochem.*, **2015**, *491*, 58.
40. K. Kuntamung, J. Jakmunee, K. Ounnunkad, *J. Mater. Chem. B* **2021**, *9*, 6576.
41. L. Wang, N. Liu, Z. Ma, *J. Mater. Chem. B* **2015**, *3*, 2867.
42. D. Du, Z. Zou, Y. Shin, J. Wang, H. Wu, M. H. Engelhard, J. Liu, I. A. Aksay, Y. Lin, *Anal. Chem.*, **2010**, *82*, 2989.
43. C. A. Amarnath, S. N. Sawant, *Electroanalysis* **2020**, *32*, 2415.
44. C. A. Amarnath, V. Geetha, M. Doble, S. N. Sawant, *Microchem. J.*, **2022**, *181*, 107819
45. C. A. Amarnath, S. N. Sawant, *Microchim. Acta* **2021**, *188*, 403.

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