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VISVESVARAYA TECHNOLOGICAL UNIVERSITY
State University of Government of Karnataka Established as per the VTU Act, 1994 "JnanaSangama"
Belagavi-590018, Karnataka, India

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REGISTRAR

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REF: VTU/BGM/BoS/ SO1/Handbooks/621/2024-25/ 2401

DATE 26 AUG 2024

CIRCULAR

Subject: Handbooks and Manual regarding...

Reference: Chairperson BoS in Biotechnology vide email dated 23.08.2024

This refers to the subject cited above, board of studies have submitted the handbooks and manual for the following courses/subjects

1. BETCK105F- Waste Management (Handbook)
2. BETCK105G - Emerging Applications of Biosensors(Handbook)
3. BBTL358C- Analysis of Diary Products (Lab Manual)

The Principals of Engineering Colleges are requested to inform the content of the circular to all concerned staff and students.

Encl: enclosed as mentioned above.

To,

1. The Principals of Engineering where Biotechnology program being offered

Copy to:

- The Hon'ble Vice-Chancellor's through the secretary to VC for information
- The Registrar(Evaluation), VTU Belagavi for information
- The Director, ITI SMU VTU Belgaum for information and request to upload the circular on VTU web portal

26/08/24

REGISTRAR

Hand Book on

Waste Management

BETC151

For First Year BE, VTU, Belgaum

Course Title:	Waste Management		
Course Code:	BETC151	CIE Marks	50
Course Type (Theory/Practical /Integrated)	Theory	SEE Marks	50
		Total Marks	100
Teaching Hours/Week (L:T:P: S)	3:0:0:0	Exam Hours	3 hrs of Theory
Total Hours of Pedagogy	40 hours	Credits	03

Course objectives

- To learn broader understandings on various aspects of solid waste management practiced in industries.
- To learn recovery of products from solid waste to compost and biogas, incineration and energy recovery, hazardous waste management and treatment, and integrated waste management.

Module-1 (08)

INTRODUCTION TO SOLID WASTE MANAGEMENT:

Classification of solid wastes (source and type based), solid waste management (SWM), elements of SWM, ESSWM (environmentally sound solid waste management) and EST (environmentally sound technologies), factors affecting SWM, Indian scenario, progress in MSW (municipal solid waste) management in India. Indian and global scenario of e-waste,

Module-2 (08)

WASTE GENERATION ASPECTS:

Waste stream assessment (WSA), waste generation and composition, waste characteristics (physical and chemical), health and environmental effects (public health and environmental), comparative assessment of waste generation and composition of developing and developed nations, a case study results from an Indian city, handouts on solid waste compositions. E-waste generation.

Module-3 (08)

COLLECTION, STORAGE, TRANSPORT AND DISPOSAL OF WASTES:

Waste Collection, Storage and Transport: Collection components, storage-containers/collection vehicles, collection operation, transfer station, waste collection system design, record keeping, control, inventory and monitoring, implementing collection and transfer system, a case study. Waste Disposal: key issues in waste disposal, disposal options and selection criteria, sanitary landfill, landfill gas emission, leachate formation, environmental effects of landfill, landfill operation issues, a case study.

Module-4 (08)

WASTE PROCESSING TECHNIQUES & SOURCE REDUCTION, PRODUCT RECOVERY & RECYCLING:

Purpose of processing, mechanical volume and size reduction, component separation, drying and dewatering. Source Reduction, Product Recovery and Recycling: basics, purpose, implementation monitoring and evaluation of source reduction, significance of recycling, planning of a recycling programme, recycling programme elements, commonly recycled materials and processes, a case study.

Module-5 (08)

HAZARDOUS WASTE MANAGEMENT AND TREATMENT:

Identification and classification of hazardous waste, hazardous waste treatment, pollution prevention and waste minimization, hazardous wastes management in India. E-waste recycling.

Course outcome (Course Skill Set)

At the end of the course the student will be able to:

CO1	Apply the basics of solid waste management towards sustainable development
CO2	Apply technologies to process waste and dispose the same.
CO3	Design working models to convert waste to energy
CO4	Identify and classify hazardous waste and manage the hazard

Module 1

INTRODUCTION TO SOLID WASTE MANAGEMENT

1.1 Classification of solid wastes (source and type based),
1.2 Solid waste management (SWM);
  1.2.1 Elements of SWM,
  1.2.2. ESSWM (environmentally sound solid waste management) and EST (environmentally sound technologies),
  1.2.3 Factors affecting SWM,
1.3 Indian scenario; progress in MSW (municipal solid waste) management in India.
1.4 Indian and global scenario of e-waste,

1.1 CLASSIFICATION OF SOLID WASTE

- Solid wastes are the organic and inorganic waste materials such as product packaging, grass clippings, furniture, clothing, bottles, kitchen refuse, paper, appliances, paint cans, batteries, etc., produced in a society, which do not generally carry any value to the first user(s).
- Solid wastes, thus, encompass both a heterogeneous mass of wastes from the urban community as well as a more homogeneous accumulation of agricultural, industrial and mineral wastes.
- While wastes have little or no value in one setting or to the one who wants to dispose them, the discharged wastes may gain significant value in another setting.
- Knowledge of the sources and types of solid wastes as well as the information on composition and the rate at which wastes are generated/ disposed is, therefore, essential for the design and operation of the functional elements associated with the management of solid wastes.

1.1.1 Solid wastes are classified on the basis of source of generation and type

Source-based classification:

The sources of solid wastes have been consistent, dependent on sectors and activities and these include the following:

- **Residential:** This refers to wastes from dwellings, apartments, etc., and consists of leftover food, vegetable peels, plastic, clothes, ashes, etc.
- **Commercial:** This refers to wastes consisting of leftover food, glasses, metals, ashes, etc., generated from stores, restaurants, markets, hotels, motels, auto-repair shops, medical facilities, etc.
- **Institutional**: This mainly consists of paper, plastic, glasses, etc., generated from educational, administrative and public buildings such as schools, colleges, offices, prisons, etc.

- **Municipal**: This includes dust, leafy matter, building debris, treatment plant residual sludge, etc., generated from various municipal activities like construction and demolition, street cleaning, landscaping, etc.
- **Industrial**: This mainly consists of process wastes, ashes, demolition and construction wastes, hazardous wastes, etc., due to industrial activities.
- **Agricultural**: This mainly consists of spoiled food grains and vegetables, agricultural remains, litter, etc., generated from fields, orchards, vineyards, farms, etc.
- **Open areas**: this includes wastes from areas such as Streets, alleys, parks, vacant lots, playgrounds, beaches, highways, recreational areas, etc.

Type-based classification: Classification of wastes based on types, i.e., physical, chemical, and biological characteristics of wastes

- Garbage
- Ashes and residues
- Combustible and non-combustible wastes:
- Bulky wastes
- Street wastes
- Biodegradable and non-biodegradable wastes:
- Dead animals
- Abandoned vehicles:
- Construction and demolition wastes:
- Hazardous wastes:
- Sewage wastes:

Fig 1: Types of Solid Waste

1.2 SOLID WASTE MANAGEMENT (SWM)

- Solid waste management (SWM) is associated with the control of waste generation, its storage, collection, transfer and transport, processing and disposal in a manner that is in accordance with the best principles of public health, economics, engineering, conservation, aesthetics, public attitude and other environmental considerations.

1.2.1 Elements of SWM system

A SWM system refers to a combination of various functional elements associated with the management of solid wastes. The system, when put in place, facilitates the collection and disposal of solid wastes in the community at minimal costs, while preserving public health and ensuring little or minimal adverse impact on the environment. The functional elements that constitute the system are:

- **Waste generation:** Wastes are generated at the start of any process, and thereafter, at every stage as raw materials are converted into goods for consumption. The source of waste generation, determines quantity, composition and waste characteristics.
- **Waste storage:** Storage is a key functional element because collection of wastes never takes place at the source or at the time of their generation... Onsite storage is of primary importance due to aesthetic consideration, public health and economics involved. Some of the options for storage are plastic containers, conventional dustbins (of households), used oil drums, large storage bins (for institutions and commercial areas or servicing depots), etc.
- **Waste collection:** This includes gathering of wastes and hauling them to the location, where the collection vehicle is emptied, which may be a transfer station, a processing plant or a disposal site. Collection depends on the number of containers, frequency of collection, types of collection services and routes.
- **Transfer and transport:** This functional element involves: the transfer of wastes from smaller collection vehicles, where necessary to overcome the problem of narrow access lanes, to larger ones at transfer stations; the subsequent transport of the wastes, usually over long distances, to disposal sites.
- **Processing**: Processing is required to alter the physical and chemical characteristics of wastes for energy and resource recovery and recycling. The important processing techniques include compaction, thermal volume reduction, and manual separation of waste components, incineration and composting.

- **Recovery and recycling**: This includes various techniques, equipment and facilities used to improve both the efficiency of disposal system and recovery of usable material and energy.
- **Waste disposal**: Disposal is the ultimate fate of all solid wastes, be they residential wastes, semi-solid wastes from municipal and industrial treatment plants, incinerator residues, composts or other substances that have no further use to the society. Thus, land use planning becomes a primary determinant in the selection, design and operation of landfill operations. A modern sanitary landfill is a method of disposing solid waste without creating a nuisance and hazard to public health.

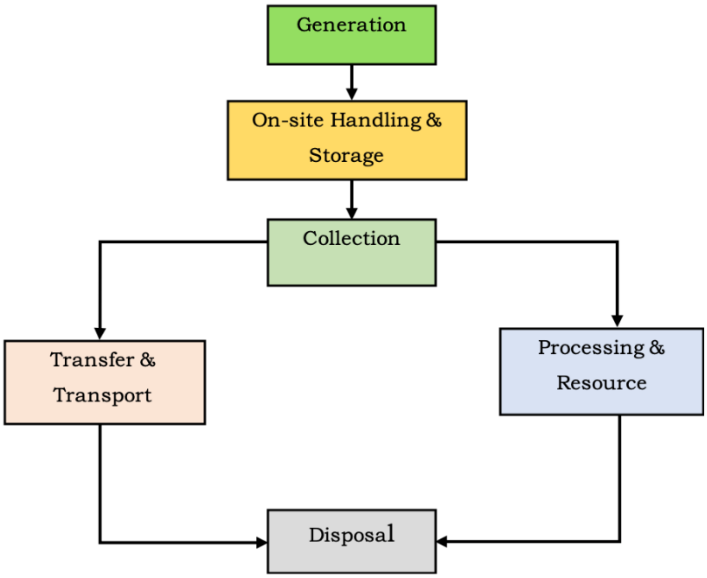

Figure 2: Elements of SWM.

1.2.2 Environmentally sound solid waste management (ESSWM):

- In any waste or resource management system, we must pay attention to the interaction between human activities and the ecosystem.
- We have to recognise that human activities including consumption of goods/services, production of wastes, etc., have a serious impact on the carrying capacity of the ecosystem.
- This in turn affects human health, as the environment deteriorates. The fundamental principles of ESSWM, which take into account economic and social issues along with environmental impact consideration, include the following:
- To ensure sustainable development of the ecosystem and human environment. To minimise the impact of human activities on the environment.
- To minimise the impact on the environment and maximise the ecosystem's carrying capacity.
- To ensure the implementation of ESSWM through environmentally sound technologies.

1.2.3 Environmentally sound technologies (EST)

EST refers to cost effective and energy efficient technologies, which generally perform better on the environment, as they do not pollute the ecosystem's vital components such as air, land or water and consider the reuse, recycling or recovery of wastes. EST can be categorised broadly as follows:

- Hard EST: This includes equipment, machines and other infrastructure with their material accessories to handle waste products and monitor/measure the quality of air, water and soil.
- Soft EST: This supports and complements hard technologies and include nature-based technologies and management tools. Nature-based technologies include processes and mechanisms nature uses within a specific ecosystem (such as vermin composting) and its carrying capacity, while management tools include system and procedures, policy and regulatory frameworks, and environmental performance standards and guidelines.

EST is selected based on the following generic criteria, the indicators of which may vary depending on the regions in which they are implemented:

- Affordability: This means low investment, reasonableness, maintenance free and durability.
- Validity: This refers to effectiveness, easy operation and maintenance.
- Sustainability: This means low impact, energy saving and cultural acceptability.

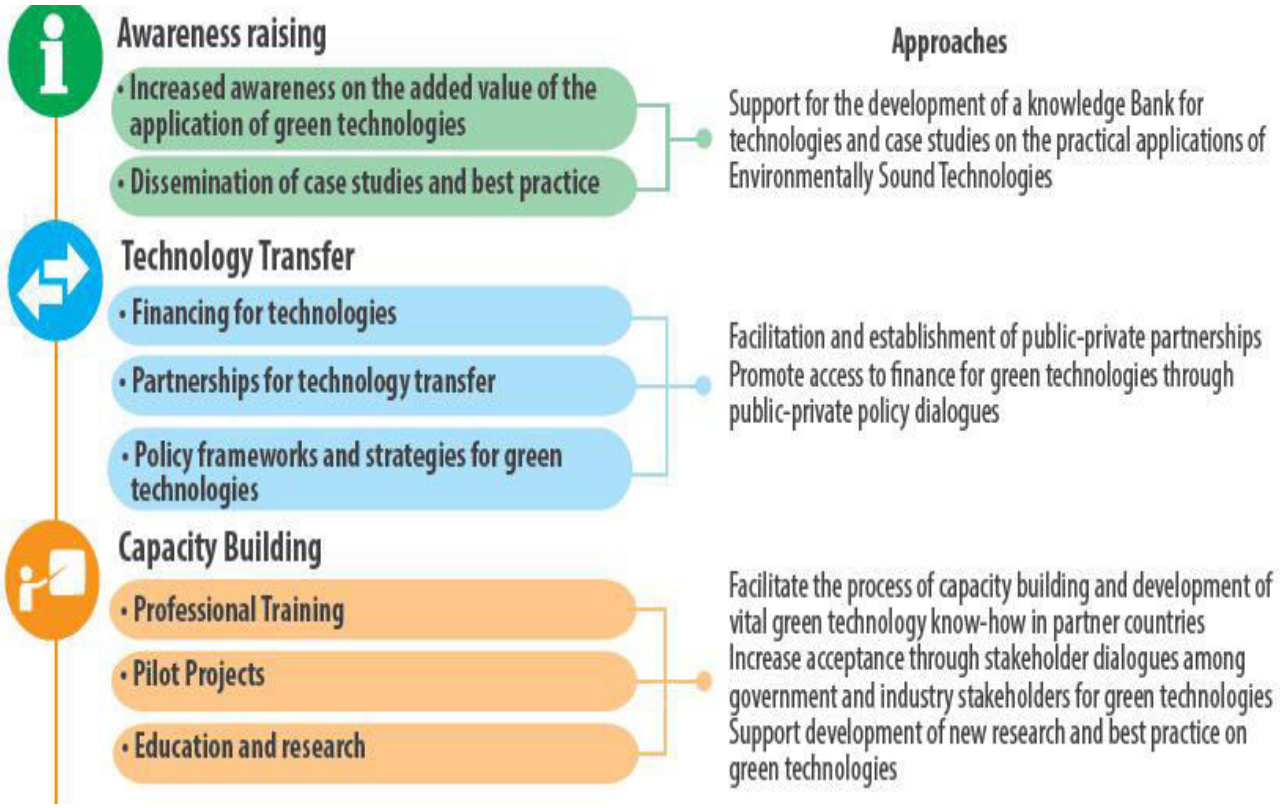

Figure 3: EST.

1.2.4 Factors affecting SWM system

Many factors influence the decision-making process in the implementation of a SWM system. Some of the factors that need to be considered in developing a SWM system are Quantities and characteristics of wastes, Climate and seasonal variations, Physical characteristics of an urban area, Financial and foreign exchange constraints, Cultural constraints, and Management and technical resources,

Quantities and characteristics of wastes:

- The quantities of wastes generated generally depend on the income level of a family, as higher income category tends to generate larger quantity of wastes, compared to low-income category.
- The quantity ranges from about 0.25 to about 2.3 kg per person per day, indicating a strong correlation between waste production and per capita income.
- One of the measures of waste composition (and characteristics) is density, which ranges from 150 kg/m^3 to 600 kg/m^3.
- Proportion of paper and packaging materials in the waste largely account for the differences.
- When this proportion is high, the density is low and vice versa.
- The wastes of high density reflect a relatively high proportion of organic matter and moisture and lower levels of recycling.

Climate and seasonal variations:

- There are regions in extreme north (>70 N Latitude) and south (> 60 S Latitude), where temperatures are very low for much of the year. In cold climates, drifting snow and frozen ground interfere with landfill operations, and therefore, trenches must be dug in summer and cover material stockpiled for winter use.
- Tropical climates, on the other hand, are subject to sharp seasonal variations from wet to dry season, which cause significant changes in the moisture content of solid waste, varying from less than 50% in dry season to greater than 65% in wet months.
- Collection and disposal of wastes in the wet months are often problematic.
- High temperatures and humidity cause solid wastes to decompose far more rapidly than they do in colder climates.
- The frequency of waste collection in high temperature and humid climates should, therefore, be higher than that in cold climates. In sub-tropical or desert climate, there is no significant variation in moisture content of wastes (due to low rainfall) and low production of leachate from sanitary landfill.

- High winds and windblown sand and dust, however, cause special problems at landfill sites.
- While temperature inversions can cause airborne pollutants to be trapped near ground level, landfill sites can affect groundwater by altering the thermal properties of the soil.

Physical characteristics of an urban area:

- In urban areas (i.e., towns and cities), where the layout of streets and houses is such that access by vehicles is possible and door-to-door collection of solid wastes is the accepted norm either by large compaction vehicle or smaller vehicle.
- The picture is, however, quite different in the inner and older city areas where narrow lanes make service by vehicles difficult and often impossible.
- Added to this is the problem of urban sprawl in the outskirts (of the cities) where population is growing at an alarming rate.
- Access ways are narrow, unpaved and tortuous, and therefore, not accessible to collection vehicles.
- Problems of solid waste storage and collection are most acute in such areas.

Financial and foreign exchange constraints:

- Solid waste management accounts for sizeable proportions of the budgets of municipal corporations.
- This is allocated for capital resources, which go towards the purchase of equipments, vehicles, and fuel and labour costs.
- Typically, 10% to 40% of the revenues of municipalities are allocated to solid waste management. In regions where wage rates are low, the aim is to optimise vehicle productivity.
- The unfavourable financial situation of some countries hinders purchase of equipment and vehicles, and this situation is further worsened by the acute shortage of foreign exchange.
- This means that the balance between the degree of mechanisation and the size of the labour force becomes a critical issue in arriving at the most cost-effective solution.

Cultural constraints:

- In some regions, long-standing traditions preclude the intrusion of waste collection on the precincts of households, and therefore, influence the collection system.
- In others, where the tradition of caste persists, recruits to the labour force for street cleaning and handling of waste must be drawn from certain sections of the population, while others will not consent to placing storage bins in their immediate vicinity.
- Social norms of a community more often than not over-ride what many may consider rational solutions.

- Waste management should, therefore, be sensitive to such local patterns of living and consider these factors in planning, design and operation.

Management and technical resources:

- Solid waste management, to be successful, requires a wide spectrum of workforce in keeping with the demands of the system.
- The best system for a region is one which makes full use of indigenous crafts and professional skills and/or ensures that training programmes are in place to provide a self-sustaining supply of trained workforce.

1.3 SWM: THE INDIAN SCENARIO

- The problem of municipal solid waste management has acquired alarming dimensions in India especially over the last decade, before which waste management was hardly considered an issue of concern as the waste could be easily disposed of in an environmentally safe manner.
- However, with time, due to changing lifestyles of people coupled with unplanned developmental activities, urbanisation and industrialisation, the waste quantity and characteristics have changed, and as a result, managing solid wastes has become torturous.
- The physical and chemical characteristics of Indian city refuse, nonetheless, show that about 80% of it is compostable and ideal for biogas generation due to adequate nutrients (NPK), moisture content of 50-55% and a carbon-to-nitrogen ratio of 25-40:1. Therefore, the development of appropriate technologies for utilisation of wastes is essential to minimise adverse health and environmental consequences

Waste Generation Statistics

Year	Per capita waste generated (g/day)	Total urban municipal waste generated (Mt/year)
1971	375	14.9
1981	430	25.1
1991	460	43.5
2000	500	48.8
2010	600	~70.2

Table 1 : Waste generation statistics

- **Waste quantum:** The per capita waste generation rate is about 500 g/day. This along with increased population has contributed to higher total waste generation quantum .During the last decade, garbage was generated in India at nearly twice the rate of the population growth. Estimates

of the solid wastes generated in Indian towns and cities range from 52,000 tonnes to 85,000 tonnes of city garbage every day.

- **Waste composition**: Studies reveal that the percentage of the organic matter has remained almost static at 41% in the past 3 decades, but the recyclables have increased from 9.56% to 17.18%. Garbage in Indian cities is estimated to contain about 45-75% biodegradable waste with 50-55% moisture; 35-45% being fruits, vegetable and food biomass; and 8-15% non-organic materials like plastic, metal, glass, stones, etc.
- **Waste disposal methods:** Waste disposal is the final stage of the waste management cycle. About 90% of the municipal waste collected by the civic authorities in India is dumped in low-lying areas outside the city/town limits, which have no provision of leachate collection and treatment, and landfill gas collection and use.
- **Recycling:** This involves collection of recyclables from various sources, which ultimately reach recycling units. It is estimated that about 40-80% of plastic waste gets recycled in India, as compared to 10-15% in the developed nations of the world. However, due to lack of suitable government policies, incentives, subsidies, regulations, standards, etc., related to recycling, this industry is still far behind its western counterparts in terms of technology and quality of manufactured goods. Nevertheless, recycling in India is a highly organised and profit-making venture, though informal in nature.
- **Health impacts:** Due to the absence of standards and norms for handling municipal wastes, municipal workers suffer occupational health hazards of waste handling. At the dumpsites in the city of Mumbai, for example, 95 workers were examined and it was found that about 80% of them had eye problems, 73% respiratory ailments, 51% gastrointestinal ailments and 27% skin lesions. Also, municipal workers and rag pickers who operate informally for long hours rummaging through waste also suffer from similar occupational health diseases ranging from respiratory illnesses (from ingesting particulates and bio-aerosols), infections (direct contact with contaminated material), puncture wounds (leading to tetanus, hepatitis and HIV infection) to headaches and nausea, etc. Studies among the 180 rag pickers at open dumps of Kolkata city reveal that average quarterly incidence of diarrhoea was 85%, fever 72% and cough and cold 63%
- **Environmental impacts:** In addition to occupational health, injury issues and environmental health also need to be mentioned in the context of waste management. Contaminated leachate and surface run-off from land disposal facilities affects ground and surface water quality. Volatile organic compounds and dioxins in air-emissions are attributed to increasing cancer incidence and psychological stress for those living near incinerators or land disposal facilities. Drain clogging

due to uncollected wastes leading to stagnant waters and subsequent mosquito vector breeding are a few of the environmental health issues, which affect the waste workers as well as the public. The pneumonic plague that broke out in November 1994 in India (Surat, Gujarat) is a typical example of solid waste mismanagement.

1.3.1 Progress of MSW management in India

- Over the years, the problems faced due to MSW were highlighted by civic and environmental activists.
- This resulted in framing rules for MSW in the year 2000which are directed by the Supreme Court and MoEF. In October 2004, specific directions to the larger cities to meet the requirements of these rules were issued by Supreme Court.
- In 2005 Ministry of Urban Development giving priority to MSWM has allocated grants to the tunes of Rs 25000 million covering 423 classes I towns as part of 12th finance committee.

1.5 Indian and global scenario of e-waste

- Global demand for electronic devices is on the rise and so is the number of used and discarded gadgets.
- Around 50 million tonnes of e-waste is generated every year, which is more than the weight of all of the commercial airplanes ever made.
- If nothing changes, it is estimated that the annual amount of e-waste could more than double by 2050.
- Out of all discarded electronics, only about 20% is recycled through organised and regulated channels, while most e-waste ends up in landfills or is managed in informal settings in a number of developing countries.
- Even in the EU, which is considered to be a global leader in e-waste recycling, only 35 percent of e-waste is reported as properly managed and recycled.
- E-waste in landfills pollutes soil and groundwater.
- The informal and unregulated management of e-waste poses a serious risk to the health and well-being of both workers and communities as a whole, due to the substantial amount of harmful components such as mercury, lead, bromine, and arsenic that our devices contain.
- For instance, long-term exposure to arsenic, which is found in the microchips of many devices such as mobile phones, may cause lung cancer, nerve damage, and a variety of skin diseases.
- Lead exposure can cause brain damage, kidney damage, blood disorders, and is particularly harmful to children.

- The Basel Convention on the Control of Transboundary Movements of Hazardous Wastes and Their Disposal, effective since 1992, outlaws the export of e-waste from developed to developing countries. Moreover, the parties to the Basel Convention adopted the Ban on Exporting Hazardous Waste to Developing Countries in 1995, which prohibits the export of hazardous wastes from the members of the EU, Organisation for Economic Co-operation and Development (OECD), and Liechtenstein to all other countries. The Ban entered into force on 5 December 2019 and 98 countries that have ratified it.
- Nonetheless, a substantial amount of obsolete electronics from the developed countries end up in developing countries.
- It is estimated that 352,474 metric tonnes of electronic waste is illegally shipped from the EU to developing countries each year, most of which is exported to the African region (Nigeria, Ghana, and Tanzania, in particular).
- Large economies such as the US, Canada, South Korea, and Japan, to name a few, still have not ratified the ban.
- Even the countries that have ratified the ban have failed to comply with its provisions. For instance, the UK, which has been a party to the Ban since 1997, is considered to be Europe’s worst offender for the illegal export of e-waste, according to a two-year study that tracked shipments from 10 European countries.
- National e-waste legislation/policy or regulation in place
- The UN has also addressed the growing problem of e-waste, often warning of a ‘tsunami of e-waste rolling out over the world.’ A number of global agencies have emphasised the importance of confronting the global e-waste challenge effectively and in a timely manner, including the International Telecommunications Union (ITU), International Labour Organisation (ILO), UN Environment Programme (UNEP), and others.
- In March 2018, a number of organisations, including the aforementioned UN agencies, signed a ‘Letter of Intent’ aimed at creating a mechanism for co-ordination and collaboration among stakeholders in tackling e-waste.
- Apart from being the basis of the problem, electronics could also be part of the solution. An enormous amount of raw materials that are actually reusable are being thrown away on a daily basis, including copper, tin, iron, aluminum, fossil fuels, titanium, gold, and silver. For example, around 32kg of gold, 3,500kg of silver, and 2,200kg of bronze was recovered from about 78,985 tonnes of electronics and used to make the medals for the 2020 Olympic and Paralympic Games in Tokyo.

Current Challenges for e-Waste Elimination:

Cost of recycling e-Waste exceeds the revenue recovered:

- In many cases, the cost of recycling e-Waste exceeds the revenue recovered from materials especially in countries with strict environment regulations.

e-Waste Dumped in poor countries:

- E-Waste mostly ends up dumped in countries where environmental standards are low or nonexistent and working conditions are poor.

Lack of Waste Removal Infrastructure:

- Most developing countries lack the waste removal infrastructure and technical capacities necessary to ensure the safe disposal of hazardous waste.

Variety of Health Problems:

- E-Waste has been linked to a variety of health problems, including cancer, neurological and respiratory disorders, and birth defects.

Indian Enforcement Agencies involved in E-waste:

- Ministry of Environment Forest and Climate Change, Government of India is responsible for identification of hazardous wastes and provides permission to exporters and importers under the Environment (protection) Act, 1986.
- Central Pollution Control Board (CPCB) was constituted under the Water (Prevention and Control of Pollution) Act, 1974.
- CPCB coordinates activities with the State Pollution Control Boards and ensures implementations of the conditions of imports.
- It also monitors the compliance of the conditions of authorization, import and export and conduct training courses for authorities dealing with management of hazardous wastes.

Module 2

WASTE GENERATION ASPECTS

WASTE GENERATION ASPECTS:

2.1 Waste stream assessment (WSA),

2.2 Waste generation and composition,

2.3 Waste characteristics (physical and chemical),

2.3 Health and environmental effects (public health and environmental),

2.4 comparative assessment of waste generation and composition of developing and developed nations, a case study results from an Indian city,

2.5 Handouts on solid waste compositions.

2.6 E-waste generation.

2.1 Waste stream assessment (WSA)

- Waste stream assessment (WSA) is a means to determine the basic aspects of quantity (i.e., the amount of waste generated in the community, both in terms of weight and volume), composition (i.e., the different components of waste stream) and sources of wastes.
- The information relating to these basic aspects of wastes is vital for making decisions about the SWM system, finance and regulations.
- Put differently, an assessment of waste stream is essential in the analyses of short and long-term problems within the local waste management system.
- It also helps in targeting waste management activities and setting goals for different elements of a waste management plan.
- Waste stream assessment, however, is not a one-time activity. It is a continuous and dynamic process, because the characteristics of wastes differ depending on the regions, communities, seasons, etc.

The reasons for the analysis of waste composition, characteristics and quantity include the following

- It provides the basic data for the planning, designing and operation of the management systems.
- An ongoing analysis of the data helps detect changes in composition, characteristics and quantities of wastes, and the rates at which these changes take place, which facilitates effective implementation of management systems.
- It quantifies the amount and type of materials suitable for processing, recovery and recycling.

- It provides information that helps in deciding appropriate technologies and equipment.
- The forecast trends assist designers and manufacturers in the production of collection vehicles and equipment suitable for future needs.

2.2 WASTE GENERATION AND COMPOSITION

- Information on waste quantity and composition is important in evaluating alternatives in terms of equipment, systems, plans and management programmes.
- For example, if wastes generated at a commercial facility consist of only paper products, the appropriate equipment are shredders and balers.
- Similarly, on the basis of quantity generated, we can plan appropriate means for separation, collection and recycling programmes.
- That is to say, the success of SWM depends on the appropriate assessment of quantity of wastes generated.

Waste generation

- Waste generation encompasses those activities in which waste, be it solid or semi-solid material, no longer has sufficient economic value for its possessor to retain it.
- The processing of raw materials is the first stage when wastes are generated, and waste generation continues thereafter at every step in the process as raw materials are converted into final products for consumption.

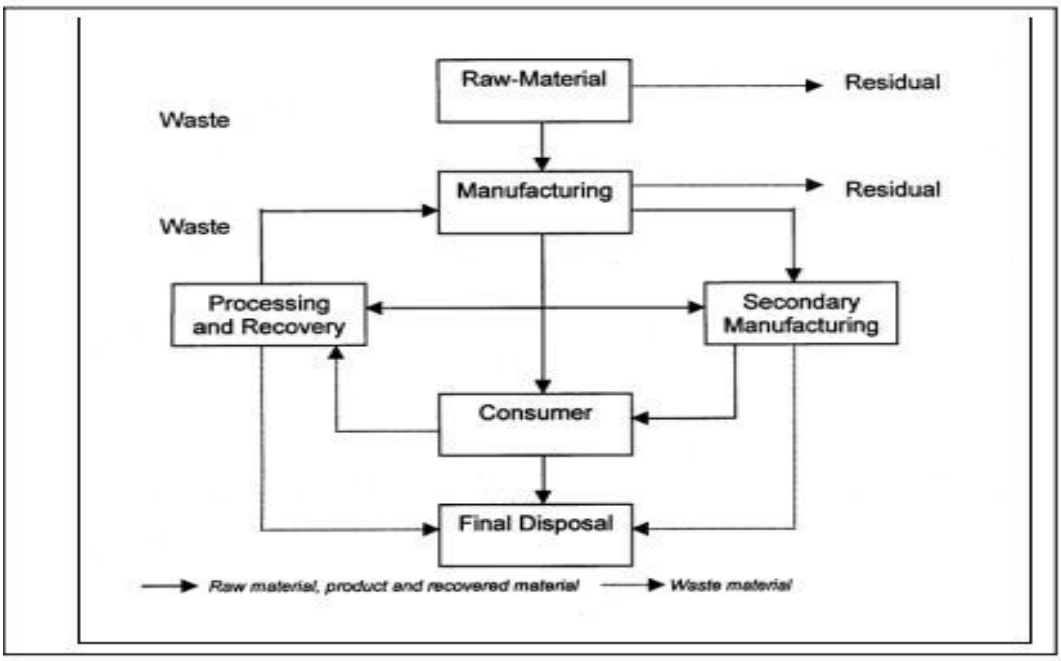

Figure 4: Simplified material-flow diagram indicating the path of generation of solid wastes

Waste composition:

Some of the general observations associated with the composition of wastes include the following:

- The major constituents are paper and decomposable organic materials.
- More often than not, metal, glass, ceramics, textile, dirt and wood form part of the composition, and their relative proportion depends on local factors.
- Average proportions of the constituents reaching the disposal sites are consistent and urban wastes are fairly constant although subject to long-term changes such as seasonal variations.
- Waste composition varies with the socio-economic status within a particular community, since income, for example, determines life style, composition pattern and cultural behaviour.
- Note that the density of waste changes as it moves from the source of generation to the point of ultimate disposal, and such factors as storage methods, salvaging activities, exposure to weather, handling methods and decomposition influence the density.
- In short, predicting changes of waste composition is as difficult as forecasting waste quantities.

2.3 WASTE CHARACTERISTICS

In order to identify the exact characteristics of municipal wastes, it is necessary that we analyse them using physical and chemical parameters.

2.3.1Physical characteristics

- Information and data on the physical characteristics of solid wastes are important for the selection and operation of equipment and for the analysis and design of disposal facilities.

The required information and data include the following:

- **Density:** Density of waste, i.e., its mass per unit volume (kg/m^3), is a critical factor in the design of a SWM system, e.g., the design of sanitary landfills, storage, types of collection and transport vehicles, etc. To explain, an efficient operation of a landfill demands compaction of wastes to optimum density. Any normal compaction equipment can achieve reduction in volume of wastes by 75%, which increases an initial density of 100 kg/m3 to 400 kg/m^3.
- **Moisture content:** Moisture content is defined as the ratio of the weight of water (wet weight - dry weight) to the total weight of the wet waste. Moisture increases the weight of solid wastes, and thereby, the cost of collection and transport. In addition, moisture content is a critical determinant in the economic feasibility of waste treatment by incineration, because wet waste consumes energy for evaporation of water and in raising the temperature of water vapour. In the main, wastes should be insulated from rainfall or other extraneous water.

- **Size:** Measurement of size distribution of particles in waste stream is important because of its significance in the design of mechanical separators and shredders. Generally, the results of size distribution analysis are expressed in the manner used for soil particle analysis. That is to say, they are expressed as a plot of particle size (mm) against percentage, less than a given value.
- The physical properties that are essential to analyse wastes disposed at landfills are: Field capacity, Permeability of compacted wastes,
- Compressibility of MSW

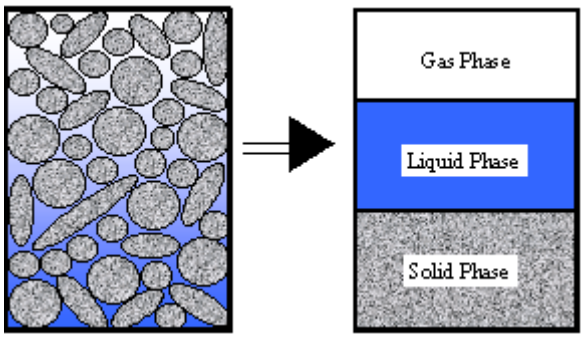

Figure 5: Geotechnical properties

2.3.2Chemical characteristics

- Knowledge of the classification of chemical compounds and their characteristics is essential for the proper understanding of the behaviour of waste, as it moves through the waste management system.
- The products of decomposition and heating values are two examples of chemical characteristics.

If solid wastes are to be used as fuel, or are used for any other purpose, we must know their chemical characteristics, including the following:

- **Lipids:** This class of compounds includes fats, oils and grease, and the principal sources of lipids are garbage, cooking oils and fats. Lipids have high heating values, about 38,000 kJ/kg (kilojoules per kilogram), which makes waste with high lipid content suitable for energy recovery. Since lipids become liquid at temperatures slightly above ambient, they add to the liquid content during waste decomposition. Though they are biodegradable, the rate of biodegradation is relatively slow because lipids have a low solubility in water.
- **Carbohydrates:** These are found primarily in food and yard wastes, which encompass sugar and polymer of sugars (e.g., starch, cellulose, etc.) with general formula (CH2O)x. Carbohydrates are readily biodegraded to products such as carbon dioxide, water and methane. Decomposing carbohydrates attract flies and rats, and therefore, should not be left exposed for long duration.

- **Proteins:** These are compounds containing carbon, hydrogen, oxygen and nitrogen, and consist of an organic acid with a substituted amine group (NH2). They are mainly found in food and garden wastes. The partial decomposition of these compounds can result in the production of amines that have unpleasant odours.
- **Natural fibres:** These are found in paper products, food and yard wastes and include the natural compounds, cellulose and lignin, that are resistant to biodegradation. (Note that paper is almost 100% cellulose, cotton over 95% and wood products over 40 %.) Because they are a highly combustible solid waste, having a high proportion of paper and wood products, they are suitable for incineration. Calorific values of oven-dried paper products are in the range of 12,000 -18,000 kJ/kg and of wood about 20,000 kJ/kg, i.e., about half that for fuel oil, which is 44,200 kJ/kg.
- **Synthetic organic material (Plastics):** Accounting for 1 – 10%, plastics have become a significant component of solid waste in recent years. They are highly resistant to biodegradation and, therefore, are objectionable and of special concern in SWM. Hence the increasing attention being paid to the recycling of plastics to reduce the proportion of this waste component at disposal sites. Plastics have a high heating value, about 32,000 kJ/kg, which makes them very suitable for incineration. But, you must note that polyvinyl chloride (PVC), when burnt, produces dioxin and acid gas. The latter increases corrosion in the combustion system and is responsible for acid rain.
- **Non-combustibles:** This class includes glass, ceramics, metals, dust and ashes, and accounts for 12 – 25% of dry solids.
- **Heating value:** An evaluation of the potential of waste material for use as fuel for incineration requires a determination of its heating value, expressed as kilojoules per kilogram (kJ/kg). The heating value is determined experimentally using the Bomb calorimeter test, in which the heat generated, at a constant temperature of 25 C from the combustion of a dry sample is measured. Since the test temperature is below the boiling point of water (100 C), the combustion water remains in the liquid state. However, during combustion, the temperature of the combustion gases reaches above 100 C, and the resultant water is in the vapour form. Table 2.3 shows the typical inert residue and heating values for the components of municipal solid waste
- **Ultimate analysis:** This refers to an analysis of waste to determine the proportion of carbon, hydrogen, oxygen, nitrogen and sulphur, and the analysis is done to make mass balance calculation for a chemical or thermal process. Besides, it is necessary to determine ash fraction because of its potentially harmful environmental effects, brought about by the presence of toxic metals such as cadmium, chromium, mercury, nickel, lead, tin and zinc. Note that other metals (e.g., iron, magnesium, etc.) may also be present but they are non-toxic

- **Proximate analysis:** This is important in evaluating the combustion properties of wastes or a waste or refuse derived fuel.

The fractions of interest are:

- **Moisture content**, which adds weight to the waste without increasing its heating value, and the evaporation of water reduces the heat released from the fuel;
- **Ash,** which adds weight without generating any heat during combustion;
- **Volatile matter**, i.e., that portion of the waste that is converted to gases before and during combustion;
- **Fixed carbon**, which represents the carbon remaining on the surface grates as charcoal. A waste or fuel with a high proportion of fixed carbon requires a longer retention time on the furnace grates to achieve complete combustion than a waste or fuel with a low proportion of fixed carbon.

2.4 Health and environmental effects

An effective solid waste management system is necessary to avoid public health disasters, spread of disease by insects and vectors and adverse effect on water and air Solid waste workers are the most exposed to the risks of parasitic infections and accidents, and therefore, a SWM system must include proper mechanisms to avoid these incidences.

2.4.1 Public health effect

- The volume of waste is increasing rapidly as a result of increasing population and improving economic conditions in various localities.
- This increased volume of wastes is posing serious problems due to insufficient workforce and other constraints in disposing of it properly.

The consequences of improper management and handling of waste include:

- **Disease vectors and pathways:** Wastes dumped indiscriminately provide the food and environment for thriving populations of vermin, which are the agents of various diseases. The pathways of pathogen transmission from wastes to humans are mostly indirect through insects – flies, mosquitoes and roaches and animals – rodents and pigs. Diseases become a public health problem when they are present in the human and animal population of surrounding communities, or if a carrier transmits the etiological agent from host to receptor.
- **Flies:** Most common in this category is the housefly, which transmits typhoid, salmonellosis, gastro-enteritis and dysentery. Flies have a flight range of about 10 km, and therefore, they are able to spread their influence over a relatively wide area. The four stages in their life-cycle are egg, larva, pupa and adult. Eggs are deposited in the warm, moist environment of decomposing food

wastes. When they hatch, the larvae feed on the organic material, until certain maturity is reached, at which time they migrate from the waste to the soil of other dry loose material before being transformed into pupae. The pupae are inactive until the adult-fly emerges. The migration of larvae within 4 to 10 days provides the clue to an effective control measure, necessitating the removal of waste before migration of larvae. Consequently, in warm weather, municipal waste should be collected twice weekly for effective control. In addition, the quality of household and commercial storage containers is very significant. The guiding principle here is to restrict access to flies. Clearly, the use of suitable storage containers and general cleanliness at their location, as well as frequent collection of wastes, greatly reduces the population of flies. Control is also necessary at transfer stations, composting facilities and disposal sites to prevent them from becoming breeding grounds for flies. Covering solid wastes with a layer of earth at landfill sites at the end of every day arrests the problem of fly breeding at the final stage.

- **Mosquitoes:** They transmit diseases such as malaria, filaria and dengue fever. Since they breed in stagnant water, control measures should centre on the elimination of breeding places such as tins, cans, tyres, etc. Proper sanitary practices and general cleanliness in the community help eliminate the mosquito problems caused by the mismanagement of solid waste.
- **Rodents:** Rodents (rats) proliferate in uncontrolled deposits of solid wastes, which provide a source of food as well as shelter. They are responsible for the spread of diseases such as plague, murine typhus, leptospirosis, histoplasmosis, rat bite fever, dalmonelosis, trichinosis, etc. The fleas, which rats carry, also cause many diseases. This problem is associated not only with open dumping but also poor sanitation
- **Occupational hazards:** Workers handling wastes are at risk of accidents related to the nature of material and lack of safety precautions. The sharp edges of glass and metal and poorly constructed storage containers may inflict injuries to workers. It is, therefore, necessary for waste handlers to wear gloves, masks and be vaccinated.

The infections associated with waste handling, include:

- Skin and blood infections resulting from direct contact with waste and from infected wounds;
- Eye and respiratory infections resulting from exposure to infected dust, especially during landfill operations;
- Diseases that result from the bites of animals feeding on the waste;
- Intestinal infections that are transmitted by flies feeding on the waste;
- Chronic respiratory diseases, including cancers resulting from exposure to dust and hazardous compounds.

2.4.2 Environmental effect

Inadequate and improper waste management causes adverse environmental effects such as the following:

- **Air pollution:** Burning of solid wastes in open dumps or in improperly designed incinerators emit pollutants (gaseous and particulate matters) to the atmosphere. Studies show that the environmental consequences of open burning are greater than incinerators, especially with respect to aldehydes and particulates. Emissions from an uncontrolled incinerator system include particulate matter, sulphur oxides, nitrogen oxides, hydrogen chloride, carbon monoxide, lead and mercury
- **Water and land pollution:** Water pollution results from dumping in open areas and storm water drains, and improper design, construction and/or operation of a sanitary landfill. Control of infiltration from rainfall and surface runoff is essential in order to minimise the production of leachate.

  Pollution of groundwater can occur as a result of:
  - the flow of groundwater through deposits of solid waste at landfill sites;
  - percolation of rainfall or irrigation waters from solid wastes to the water table;
  - Diffusion and collection of gases generated by the decomposition of solid wastes.
- **Noise pollution:** Undesirable noise is a nuisance associated with operations at landfills, incinerators, transfer stations and sites used for recycling. This is due to the movement of vehicles, the operation of large machines and the diverse operations at an incinerator site. The impacts of noise pollution may be reduced by careful siting of SWM operations and by the use of noise barriers.
- **Odour pollution:** Obnoxious odours due to the presence of decaying organic matter are characteristic of open dumps. They arise from anaerobic decomposition processes and their major constituents are particularly offensive. Proper landfill covering eliminates this nuisance.
- **Explosion hazards:** Landfill gas, which is released during anaerobic decomposition processes, contains a high proportion of methane (35 – 73%). It can migrate through the soil over a considerable distance, leaving the buildings in the vicinity of sanitary landfill sites at risk, even after the closure of landfills. Several methods are available for control of landfill gas, such as venting, flaring and the use of impermeable barriers

2.5 Comparative assessment of waste generation and composition of developing and developed nations

CASE STUDY: STATUS OF WASTE GENERATION IN BANGALORE

- Bangalore, also known as the Garden City, is one of the fastest growing metropolitan cities in South India.
- It is the state capital of Karnataka and the sixth largest city in India.
- Topographically, Bangalore is located in the south Deccan and physically, has grown on watershed running through the middle of the Mysore Plateau from west to east which serves as the main water parting of the state at an average elevation of 900 meters above sea level.
- The city gets moderate rainfall of around 900 mm largely between June and October. On account of its elevation,
- Bangalore is bestowed with salubrious and equable climate comparable to those of temperate regions.

The waste generation and composition details of Bangalore are as follows:

- **Waste generation:** Bangalore produces over 2500 tonnes of solid waste per day and the Municipal Corporation has miserably inadequate infrastructure in managing the disposal of solid wastes generated. It is estimated that the per capita generation of solid waste works out to 0.59 kg/day. The sources of waste generation and the amount generated at each source are given in Table

Sl No.	Source	Quantity (in MT/day)
1	Households	1000
2	Shops, Establishments, Institutions, etc.	600
3	Markets	600
4	Others	300
5	TOTAL	2500

Table 2. Different sources of solid waste generation in Bengaluru

- **Waste composition:** The composition of wastes in Bangalore has wide variations in the proportion of contents. It varies from area to area, depending upon the socio-economic conditions and the population density. The composition of the total wastes generated in Bangalore city is given in Table

Sl No.	Type of Waste	Composition (in percentage)
1	Putrescible waste	75.2
2	Dust and ash	12
3	Textiles	3.1
4	Paper	1.5
5	Plastic, leather and rubber	0.9
6	Glass	0.2
7	Metals	0.1
8	Earth and building debris and others	0.7

Table 3. Composition of solid waste in Bengaluru

2.3 E-waste generation

- The major problem associated with e-waste management is its ever increasing quantum.
- However, the e-waste quantities represent a small percentage of the overall municipal solid waste (MSW).
- Data on e-waste generation may vary between areas of a country because of the definitions of waste arising, technological equipment used, the consumption patterns of the consumers, and changes in the living standards across the globe.
- Global e-waste generated per year amounts to approximately 20-25 million tons, most of which is being produced in rich nations such as the United States (US) or European Union member countries.
- The US, is the largest generator of e-waste, with a total accumulation of 3 million tons per year; and China is the second largest, producing 2.3 million tons each year.
- Brazil generates the second greatest quantity of e-waste among emerging countries.
- In Malaysia, the volume of e-waste generated is estimated at roughly 0.8-1.3 kg of waste per capita per day, with an increasing trend of e-waste generation, which rose to 134,000 tons in 2009. Furthermore, the volume of e-waste in Malaysia is expected to rise to 1.1 million metric tons in 2020, at an annual rate of 14%.
- In South Africa and China, e-waste production from old computers will increase by 200-400% from 2007 to 2020, and by 500% in India.
- In this same period e-waste from televisions will be 1.5-2 times higher in China and India; whereas in India, e-waste from discarded refrigerators will double or triple by 2020.
- For India, the volume of e-waste generated is 146,000 tonnes per year. The rate at which the e-waste volume is increasing globally is 5 to 10% yearly.

Module 3

COLLECTION, STORAGE, TRANSPORT AND DISPOSAL OF WASTES:

3.1 Waste Collection, Storage and Transport:
- 3.1.1 Collection Components,
- 3.1.2 Storage-Containers/Collection Vehicles,
- 3.1.3 Collection Operation,
- 3.1.4 Transfer Station,
- 3.1.5 Waste Collection System Design,
- 3.1.6 Record Keeping, Control, Inventory And Monitoring,
- 3.1.7 Implementing, Collection and Transfer System, A Case Study.

3.2 Waste Disposal:
- 3.2.1 Key Issues In Waste Disposal,
- 3.2.2 Disposal Options And Selection Criteria,
- 3.2.3 Sanitary Landfill,
- 3.2.4 Landfill Gas Emission,
- 3.2.5 Leachate Formation,
- 3.2.6 Environmental Effects Of Landfill, Landfill Operation Issues, A Case Study

3.1 WASTE COLLECTION, STORAGE AND TRANSPORT:

3.1.1 COLLECTION COMPONENTS

- Waste collection does not mean merely the gathering of wastes, and the process includes, as well, the transporting of wastes to transfer stations and/or disposal sites.

To elaborate, the factors that influence the waste collection system include the following

- **Collection points:** These affect such collection system components as crew size and storage, which ultimately control the cost of collection. Note that the collection points depend on locality and may be residential, commercial or industrial.
- **Collection frequency:** Climatic conditions and requirements of a locality as well as containers and costs determine the collection frequency. In hot and humid climates, for example, solid wastes

must be collected at least twice aweek, as the decomposing solid wastes produce bad odour and leachate.

. While deciding collection frequency, therefore, you must consider the following:

- Cost, e.g., optimal collection frequency reduces the cost as it involves fewer trucks, employees and reduction in total route distance;
- Storage space, e.g., less frequent collection may require more storagespace in the locality;
- Sanitation, e.g., frequent collection reduces concerns about health, safety and nuisance associated with stored refuse.

- **Storage containers:** Proper container selection can save collection energy, increase the speed of collection and reduce crew size. Most importantly, containers should be functional for the amount and type of materials and collection vehicles used.
- **Collection crew** the optimum crew size for a community depends on labour and equipment costs, collection methods and route characteristics. The size of the collection crew also depends on the size and type of collection vehicle used, space between the houses, waste generation rate and collection frequency. For example, increase in waste generation rate and quantity of wastes collected per stop due to less frequent collection result in a bigger crew size.

  However, with increase in collection costs, the trend in recent years is towards:

  - Decrease in the frequency of collection;
  - Increase in the dependence on residents to sort waste materials;
  - Increase in the degree of automation used in collection.
  - This trend has, in fact, contributed to smaller crews in municipalities.
- **Collection route: The** collection programmemust consider the route that is efficient for collection. An efficient routing of collection vehicles helps decrease costs by reducing the labour expended for collection. Proper planning of collection route also helps conserve energy and minimise working hours and vehicle fuel consumption. It is necessary therefore to develop detailed route configurations and collection schedules for the selected collection system. The size of each route, however, depends on the amount of waste collected per stop, distance between stops, loading time and traffic conditions. Barriers, such as railroad, embankments, rivers and roads with heavy traffic, can be considered to divide route territories.

3.1.2 STORAGE: CONTAINERS/COLLECTION VEHICLES:

- Waste storage is an important component of a waste management system.
- Waste storage encompasses proper containers to store wastes and efficient transport of wastes

without any spillage to transfer stations/disposal sites.

Containers/storage bins

- The design of an efficient waste collection system requires careful consideration of the type, size and location of containers at the point of generation for storage of wastes until they are collected. While single-family households generally use small containers, residential units, commercial units, institutions and industries require large containers. Smaller containers are usually handled manually whereas the larger, heavier ones require mechanical handling. The containers may fall under either of the following two categories:
- Stationary containers: These are used for contents to be transferred to collection vehicles at the site of storage.
- Hauled containers: These are used for contents to be directly transferred to a processing plant, transfer station or disposal site for emptying before being returned to the storage site.
- The desirable characteristics of a well-designed container are low cost, size, weight, shape, resistance to corrosion, water tightness, strength and durability. For example, a container for manual handling by one person should not weigh more than 20 kg, lest it may lead to occupational health hazards such as muscular strain, etc. Containers that weigh more than 20 kg, when full, require two or more crew members to manually load and unload the wastes, and which result in low collection efficiency.

Communal Containers

- Generally, the containers used for waste storage are communal/public containers.
- Figure below shows a typical communal container, which a compactor collection vehicle can lift and empty mechanically:

Fig 6. Typical Communal Container

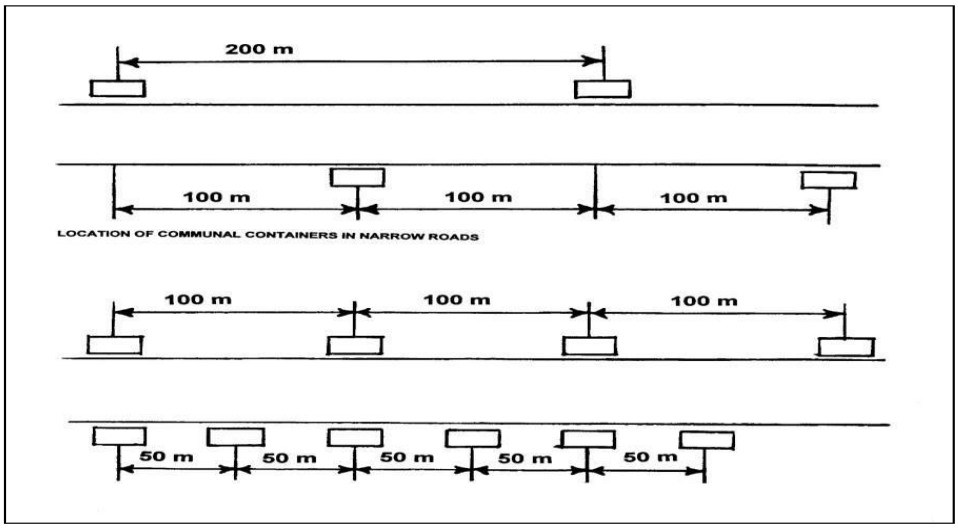

Fig 7 Location of Communal Container

- This means that the farthest distance the householder will have to walk is 50 meters.
- However, in narrow streets with low traffic, where the house owner can readily cross the street, a longer distance is advisable.
- If the collection vehicle has to stop frequently, say, at every 50 m or so, fuel consumption increases, and this must be avoided.

Collection vehicles

- Almost all collections are based on collector and collection crew, which move through the collection service area with a vehicle for collecting the waste material.
- The collection vehicle selected must be appropriate to the terrain, type and density of waste generation points, the way it travels and type and kind of material.
- It also depends upon strength, stature and capability of the crew that will work with it.
- The collection vehicle may be small and simple (e.g., two-wheeled cart pulled by an individual) or large, complex and energy intensive (e.g., rear loading compactor truck).
- The most commonly used collection vehicle is the dump truck fitted with a hydraulic lifting mechanism.
- A description of some vehicle types follows:
- **Small-scale collection and muscle-powered vehicles:** These are common vehicles used for waste collection in many countries and are generally used in rural hilly areas. As Figure 3.3 illustrates, these can be small rickshaws, carts or wagons pulled by people or animals, and are less expensive, easier to build and maintain compared to other vehicles:

- They are suitable for densely populated areas with narrow lanes, and squatter settlements, where there is relatively low volume of waste generated.
- Some drawbacks of these collection vehicles include limited travel range of the vehicles and weather exposure that affect humans and animals.
- **Non-compactor trucks:** Non-compactor trucks are efficient and cost effective in small cities and in areas where wastes tend to be very dense and have little potential for compaction. Figure 3.4 illustrates a non- compactor truck:
- When these trucks are used for waste collection, they need a dumping system to easily discharge the waste.
- It is generally required to cover the trucks in order to prevent residue flying off or rain soaking the wastes.
- Trucks with capacities of 10 – 12 m^3 are effective, if the distance between the disposal site and the collection area is less than 15 km.
- If the distanceis longer, a potential transfer station closer than 10 km from the collection area is required.
- Non-compactor trucks are generally used, when labour cost is high. Controlling and operating cost is a deciding factor, when collection routes are long and relatively sparsely populated.
- **Compactor truck:** Compaction vehicles are more common these days, generally having capacities of 12 – 15 m^3 due to limitations imposed by narrow roads.
- Although the capacity of a compaction vehicle, illustrated in Figure 3.4, is similar to that of a dump truck, the weight of solid wastes collected per trip is 2 to 2.5 times larger since the wastes are hydraulically compacted.
- Success of waste management depends on the level of segregation at source. One of the examples for best collection method is illustrated in the figure below

3.1.3 COLLECTION OPERATION

Movement of Collection Crew

- In cultures such as India, Bangladesh, etc., solid waste collection is assigned to the lowest social group.
- More often, the collection crew member accepts the job as a temporary position or stopgap arrangement, while looking for other jobs that are considered more respectable.
- Apart from this cultural problem, the attitude of some SWM authorities affects collection operation. For example, some authorities still think that the collection ofsolid waste is mechanical,

and therefore, the collection crew does not need any training to acquire special skills.

- As a result, when a new waste collector starts working, he or she is sent to the field without firm instruction concerning his or her duties, responsibilities and required skills.
- For an effective collection operation, the collection team must properly be trained.
- The collection crew and the driver of the collection vehicle must, for example, work as a team, and this is important to maintain the team morale and a sense of social responsibility amongthese workers.

Collection Vehicle Routing

- Efficient routing and re-routing of solid waste collection vehicles can help decrease costs by reducing the labour expended for collection. Routing procedures usually consist of the following two separate components:
- **Macro-routing:** Macro-routing, also referred to as route-balancing, consists of dividing the total collection area into routes, sized in such a way as to represent a day’s collection for each crew. The size of each route depends on the amount of waste collected per stop, distance between stops, loading time and traffic conditions. Barriers, such as railroad embankments, rivers and roads with heavy competing traffic, can be used to divide route territories. As much as possible, the size and shape of route areas should be balanced within the limits imposed by such barriers.
- **Micro-routing:** Using the results of the macro-routing analysis, micro- routing can define the specific path that each crew and collection vehicle will take each collection day. Results of micro-routing analyses can then be used to readjust macro-routing decisions. Micro-routing analyses should also include input and review from experienced collection drivers.
- Districting is the other method for collection route design.
- For larger areas it is not possible for one institution to handle it then the best way is to sub divide the area and MSW collection districting plan can be made.
- This routing will be successful only when road network integrity is good and the regional proximity has been generated.
- The heuristic (i.e., trial and error) route development process is a relatively simple manual approach that applies specific routing patterns to block configurations.
- The map should show collection, service garage locations, disposal or transfer sites, one-way streets, natural barriers and areas of heavy traffic flow.
- Routes should then be traced onto the tracing paper using the following rules:
- Routes should not be fragmented or overlapping. Each route should be compact, consisting of street segments clustered in the same geographical area.

- Total collection plus hauling time should be reasonably constant for each route in the community.
- The collection route should be started as close to the garage or motor pool as possible, taking into account heavily travelled and one-way streets.
- Heavily travelled streets should not be visited during rush hours.
- In the case of one-way streets, it is best to start the route near the upper end of the street, working down it through the looping process.
- Services on dead-end streets can be considered as services on the street segment that they intersect, since they can only be collected by passing down that street segment.
- To keep right turns at a minimum, (in countries where driving is left-oriented) collection from the dead-end streets is done when they are to the left of the truck.
- They must be collected by walking down, reversing the vehicle or taking a U-turn.
- Waste on a steep hill should be collected, when practical, on both sides of the street while vehicle is moving downhill. This facilitates safe, easy and fast collection. It also lessens wear of vehicle and conserves gas and oil.
- Higher elevations should be at the start of the route.
- For collection from one side of the street at a time, it is generally best to route with many anti-clockwise turns around blocks.
- For collection from both sides of the street at the same time, it is generally best to route with long, straight paths across the grid before looping anti- clockwise.
- For certain block configurations within the route, specific routing patterns should be applied. (Adapted from American Public Works Association, 1975.)

3.1.4 TRANSFER STATIONS

- A transfer station is a building or processing site for the temporary deposition of waste. Transfer stations are often used as places where local waste collection vehicles will deposit their waste cargo prior to loading into larger vehicles.
- Typical activities at the waste transfer station involved the unloading of garbage trucks, pre-screening and removal of inappropriate items such as automobile batteries, compacting and then reloading onto larger vehicles, including trucks, trains and barges to their final destination.
- The transfer station is a key component of cost-effective solid waste transportation. By transferring waste from local collection vehicles onto larger trailers or other transport modes such as barge and rail, the cost of transportation to distant disposal sites can be significantly reduced, freeing collection-specific vehicles and crews to devote their time to actual collection activities. Here are some of the main benefits:

- Provides fuel savings, reduction in road wear and less air pollution due to fewer vehicles being on the road
- Provides a trash and recyclable material drop-off location for citizens
- Reduces total traffic congestion in the community by transferring it onto larger vehicles
- Reduces total truck traffic and improves safety at the landfill or waste-to-energy facility
- Provides the opportunity to screen incoming trash for such purposes as removing hazardous waste or recovering recyclables

3.1.5 WASTE COLLECTION SYSTEM DESIGN

- After we identify appropriate options for collection, equipment and transfer, we must examine the various combinations of these elements to define system -wide alternatives for further analysis.
- Each should be evaluated for its ability to achieve the identified goals of the collection programme.
- Economic analysis will usually be a central focus of the system evaluation.
- This initial evaluation will lead to several iterations, with the differences between the alternatives under consideration becoming more narrowly focused with each round of evaluations.
- After comparing the alternative strategies, the various elements like crew and truck requirement, time requirement and cost involved are calculated.
- The various formulae used to calculate are:

  Number of services/vehicle load (N):

  $N = (C \times D)/W$

  where, C = Vehicle capacity (m^3); D = Waste density (kg/m^3)and W = Waste generation/residence (kg/service)

  Time required collecting one load (E):

  $E = N \times L$

  where, L = Loading time/residence, including on-route travel

  Number of loads/crew/day (n):

  The number of loads (n) that each crew can collect in a day can be estimated based on the workday length (t), and the time spent on administration and breaks (t_1), time for hauling and other travel (t_2) and collection route time (t)

Administrative and break time (t_1):

$t_1 = A + B$

where, A = Administrative time (i.e., for meetings, paperwork,unspecified slack time) and B = Time for breaks and lunch

Hauling and other travel time (t_2):

$t_2 = (n \times H) - f + G + J$

where, n = Number of loads/crew/day; H = Time to travelto disposal site, empty truck, and return to route; f = Timeto return from site to route; G = Time to travel from staging garage to route and J = Time to return from disposal site to garage.

Time spent on collection route (t_3):

$t_3 = n \times E$

where variables have been previously defined.

Length of workday (t):

$t = t_1 + t_2 + t_3$

where t is defined by work rules and equations A through D are solvedto find n.

Calculation of number of vehicles and crews (K):

$K = (S \times F)/(N \times n \times M)$

where, S = Total number of services in the collection area; F = Frequency of collection (numbers/week) and M =Number of workdays/week

Calculation of annual vehicle and labour costs:

Vehicle costs = Depreciation + Maintenance + Consumables + Overhead + License + Fees + Insurance

Labour costs = Drivers salary + Crew salaries + Fringe benefits + Indirect labour + Supplies + Overhead.

3.1.6 RECORD KEEPING, CONTROL, INVENTORYAND MONITORING

- For effective waste collection and, indeed, SWM, we must maintain records on the quantities of wastes collected and their variation within a week, month and year, as well as on established long-term trends in solid waste generation rates and composition, sources of wastes and the personnel collecting them.
- Long- term trends in solid waste generation rates and composition form the basis for planning, especially in budgeting for future vehicle requirements, allocating the collection vehicles and crew, building transfer stations, acquiring strategic lands and determining disposal options. Table below contains an illustration of a checklist of factors that affect the waste collection system:

Checklist of Variables Affecting Collection System

Components	Factors to Consider	
Crew size	• Labour cost • Distance between containerssize and types of containers • Loading accessories available in thetruck • Collection vehicle used	
Container type	• Solid wastes generation rate density of waste generation street width • Traffic volume • Collection crew configurationstandard of living	
Collection accessory	• Labour cost • Protection of worker's health	
Vehicle size/type	• Street width, traffic volume solid waste generation ratescrew size • Viability of a transfer station	
Collection route	• Street width, traffic volumedirection of traffic flow • Solid waste generation ratesspatial distribution of wastes • Local topography	
Transfer station	• Distance between disposal site andcollection area • Hauling cost for small and large trucks • Cost of transferring the solid wastesfrom small to large trucks	

Table 4: Checklist of factors that affect the waste collection system

3.1.7 IMPLEMENTING COLLECTION AND TRANSFER SYSTEM

- Implementing of collection and transfer system involves the following activities,which are important for success of the plan:

Finalising and implementing the system management plan:

- For proper implementation of collection and transfer system, it is necessary to have clear organisational structures and management plans.
- The organisational structure should be simple, with a minimum of administrative and management layers between collection crews and top management. All workers in the department should clearly understand the department's mission and their roles.

Purchasing and managing equipment:

- For purchasing equipment, most municipalities issue bid specifications.
- Detailed specifications include exact requirements for equipment sizes and capacities, power ratings, etc.
- Performance specifications often request that equipment be equivalent to certain available models and meet standards for capacity, speed, etc.

- In addition, each vehicle should have an individual maintenance record that includes the following items:
  - Preventive maintenance schedule;
  - Current list of specific engine;
  - Description of repairs and
  - List containing information on the repair date, mechanic, cost, type and manufacturer of repair parts and the length of time the truck was out of service, for each maintenance event.

Hiring and training personnel:

- As in all organisations, good personnel management is essential to an efficient, high-quality waste collection system.
- Authorities responsible for SWM should, therefore, strive to hire and keep well-qualified personnel.
- The recruitment programme should assess applicants' abilities to perform the types of physical labour required for the collection, equipment and methods used.
- To retain employees, management should provide a safe working environment that emphasises career advancement, participatory problem solving and worker incentives.
- Worker incentives should be developed to recognise and reward outstanding performance by employees.
- Ways to accomplish motivation include merit-based compensation, awards programme and a work structure. Feedback on employee performance should be regular and frequent.

Providing public information:

- Maintaining good communication with the public is important to a well-run collection system.
- Residents can greatly influence the performance of the collection system by co-operating in separation requirements, and by keeping undesirable materials from entering the collected waste stream.
- Commonly used methods of communicating information include brochures, articles in community newsletters, newspaper articles, announcements, and advertisements on radio and television, information attachments to utility bills (either printed or given separately) and school handouts.

Monitoring system cost and performance:

- Collection and transfer facilities should develop and maintain an effective system for cost and performance reporting. Each collection crew should complete a daily report containing the following information:
  - Total quantity hauled.

- Total distance and travel times to and from the disposal site.
- Amounts delivered to each disposal, transfer, or processing facility.
- Number of loads hauled.
- Vehicle or operational problems needing attention.

CASE STUDY:

- In the Bangalore city in India the waste collected through street sweeping is the main system of primary collection of wastes.
- However, recently efforts are being made for doorstep collection of waste through NGOs (Non-Governmental Organizations) and private contractors, but only about 5% of the population is covered under this system.
- The waste generated by the rest is collected from either the street or the dustbins.
- Other details regarding the collection process in Bangalore are given below:
- **Waste storage:** There are about 14,000 bottomless cement bins having
- 0.9 meters diameter and 0.6 cubic meter storage capacity and large masonry bins for depositing wastes at a distance of about 100 to 200 meters. Besides these, there are 1500 places, where the waste is deposited but no bins are kept on these sites. Recently, metal containers have been placed and at present 55 metal containers are in the city for the storage of waste in a more hygienic manner.

3.2 WASTE DISPOSAL

3.2.1 KEY ISSUES IN WASTE DISPOSAL

- Let us first get one thing very clear: there is no option but to dispose of wastes.
- Disposal is the final element in the SWM system.
- It is the ultimate fate of all solid wastes, be they residential wastes collected and transported directly to a landfill site, semisolid waste (sludge) from municipal and industrial treatment plants, incinerator residue, compost or other substances from various solid waste processing plants that are of no further use to society.
- It is, therefore, imperative to have a proper plan in place for safe disposal of solid wastes, which involves appropriate handling of residual matter after solid wastes have been processed and the recovery of conversion products/energy has been achieved.

  Issues to be overcome

  - To achieve effective waste disposal, we must overcome the following the constraints:
  - Municipal capacities:

- Political commitment:
- Finance and cost recovery:
- Technical guidelines:
- Location:

3.2.2 DISPOSAL OPTIONS AND SELECTION CRITERIA

Uncontrolled dumping or non-engineered disposal:

- As mentioned, this is the most common method being practiced in many parts of the world, and India is no exception.
- In this method, wastes are dumped at a designated site without any environmental control.
- They tend to remain there for a long period of time, pose health risks and cause environmental degradation.
- Due to the **Refuse-derived fuel (RDF):** adverse health and environmental impact associated with it, the non-engineered disposal is not considered a viable and safe option.

Sanitary landfill:

- Unlike the non-engineered disposal, sanitary landfill is a fully engineered disposal option in that the selected location or wasteland is carefully engineered in advance before it is pressed into service.
- Operators of sanitary landfills can minimize the effects of leachate (i.e., polluted water which flows from a landfill) and gas production through proper site selection, preparation and management.

Composting: This is a biological process of decomposition in which organisms, under controlled conditions of ventilation, temperature and moisture, convert the organic portion of solid waste into humus-like material.

- If this process is carried out effectively, what we get as the final product is a stable, odour-free soil conditioner.

Incineration: This refers to the controlled burning of wastes, at a high temperature (roughly 1200 – 1500 C), which sterilises and stabilises the waste in addition to reducing its volume.

- This is the combustible part of raw waste, separated for burning as fuel.
- Various physical processes such as screening, size reduction, magnetic separation, etc., are used to separate the combustibles.

Pyrolysis:

- This is the thermal degradation of carbonaceous material to gaseous, liquid and solid fraction in the absence of oxygen. This occurs at a temperature between 200 and 900 C.

3.2.3 SANITARY LANDFILL

- The term landfill generally refers to an engineered deposit of wastes either in pits/trenches or on the surface.
- And, a sanitary landfill is essentially a landfill, where proper mechanisms are available to control the environmental risks associated with the disposal of wastes and to make available the land, subsequent to disposal, for other purposes.
- However, you must note that a landfill need not necessarily be an engineered site, when the waste is largely inert at final disposal, as in rural areas, where wastes contain a large proportion of soil and dirt.
- This practice is generally designated as non-engineered disposal method.
- When compared to uncontrolled dumping, engineered landfills are more likely to have pre-planned installations, environmental monitoring, and organised and trained workforce.
- Sanitary landfill implementation, therefore, requires careful site selection, preparation and management.

3.2.4 LANDFILL GAS AND LEACHATE

- Leachate and landfill gas comprise the major hazards associated with a landfill. While leachate may contaminate the surrounding land and water, landfill gas can be toxic and lead to global warming and explosion leading to human catastrophe.
- The factors, which affect the production of leachate and landfill gas, are the following:

Nature of waste:

- The deposition of waste containing biodegradable matter invariably leads to the production of gas and leachate, and the amount depends on the content of biodegradable material in the waste.

Moisture content:

- Most micro-organisms require a minimum of approximately 12% (by weight) moisture for growth, and thus the moisture content of landfill waste is an important factor in determining the amount and extent of leachate and gas production.

pH:

- The methanogen bacteria within a landfill produce methane gas, which will grow only at low pH range around neutrality.

Particle size and density:

- The size of waste particle affects the density that can be achieved upon compaction and affects the surface area and hence volume.
- Both affect moisture absorption and therefore are potential for biological degradation.

Temperature:

- An increase in temperature tends to increase gas production. The temperature affects the microbial activity to the extent that it is possible to segregate bacteria, according to their optimum temperature operating conditions.

3.2.5 LANDFILL GAS EMISSION

- Landfill gas contains a high percentage of methane due to the anaerobic decomposition of organic matter, which can be utilised as a source of energy

Composition and properties

- **Methane:** This is a colourless, odourless and flammable gas with a density lighter than air, typically making up 50 – 60% of the landfill gas.
- **Carbon dioxide:** This is a colourless, odourless and non-inflammable gas that is denser than air, typically accounting for 30 – 40%.
- **Oxygen:** The flammability of methane depends on the percentage of oxygen. It is, therefore, important to control oxygen levels, where gas abstraction is undertaken.
- **Nitrogen:** This is essentially inert and will have little effect, except to modify the explosive range of methane.

Hazards of landfill gas emission

- **Explosion and fire:** Methane is flammable in air within the range of 5 – 15% by volume, while hydrogen is flammable within the range of 4.1 – 7.5% (in the presence of oxygen) and potentially explosive.
- Fire, occurring within the waste, can be difficult to extinguish and can lead to unpredictable and uncontrolled subsidence as well as production of smoke and toxic fumes.
- **Trace components:** These comprise mostly alkanes and alkenes, and their oxidation products such as aldehydes, alcohols and esters.
- Many of them are recognised as toxicants, when p present in air at concentrations above occupational exposure standards.
- **Global warming:** Known also as greenhouse effect, it is the warming of the earth's atmosphere by the accumulation of gases (methane, carbon dioxide and chlorofluorocarbons) that absorbs reflected solar radiation

3.2.6 LEACHATE FORMATION

- Leachate can pollute both groundwater and surface water supplies.
- The degree of pollution will depend on local geology and hydrogeology, nature of waste and the proximity of susceptible receptors. Once groundwater is contaminated, it is very costly to clean it up.

- Landfills, therefore, undergo siting, design and construction procedures that control leachate migration.

Composition and properties

- Leachate comprises soluble components of waste and its degradation products enter water, as it percolates through the landfill.
- The amount of leachate generated depends on: Water availability; landfill surface condition; refuse state; condition of surrounding strata.

Leachate migration

- It is generally difficult to predict the movement of escaped leachate accurately. The main controlling factors are the surrounding geology and hydrogeology.
- Escape to surface water may be relatively easy to control, but if it escapes to groundwater sources, it can be very difficult both to control and clean up.
- The degree of groundwater contamination is affected by physical, chemical and biological actions.
- The relative importance of each process may change, however, if the leachate moves from the landfill to the sub-surface region.

Control

- The best way to control leachate is through prevention, which should be integral to the site design.
- In most cases, it is necessary to control liquid access, collection and treatment, all of which can be done using the following landfill liners:
- **Natural liners:** These refer to compacted clay or shale, bitumen or soil sealants, etc., and are generally less permeable, resistant to chemical attack and have good sorption properties.
- They generally do not act as true containment barriers, because sometimes leachate migrates through them.
- **Synthetic (geo-membrane) liners**: These are typically made up of high or medium density polyethylene and are generally less permeable, easy to install, relatively strong and have good deformation characteristics.
- They sometimes expand or shrink according to temperature and age.

3.2.7 ENVIRONMENTAL EFFECTS OF LANDFILL,

- The environmental effects of a landfill include wind-blown litter and dust, noise, obnoxious odour, vermin and insects attracted by the waste, surface runoff and in aesthetic conditions.
- Gas and leachate problems also arise during the operation phase and require significant environmental controls.
- Some of the major environmental effects below:

- Wind-blown litter and dust are continuous problems of the ongoing landfill operation and a nuisance to the neighborhood.
- Covering the waste cells with soil and spraying water on dirt roads and waste in dry periods, in combination with fencing and movable screens, may minimize the problem of wind-blown litter and dust. However, note that the problem will remain at the tipping front of the landfill.
- Movement of waste collection vehicles, emptying of wastes from them, compactors, earthmoving equipment, etc., produce noise. Improving the technical capability of the equipment, surrounding the fill area with soil embankments and plantations, limiting the working hours and appropriately training the workforce will help minimize noise pollution.
- Birds (e.g., scavengers), vermin, insects and animals are attracted to the landfill for feeding and breeding. Since many of these may act as disease vectors, their presence is a potential health problem.
- Surface run-off, which has been in contact with the land filled waste, may be a problem in areas of intense rainfall. If not controlled, heavily polluted run-off may enter directly into creeks and streams. Careful design and maintenance of surface drains and ditches, together with a final soil cover on completed landfill sections, can help eliminate this problem.
- An operating landfill, where equipment and waste are exposed, appears in aesthetic. This problem may be reduced by careful design of screening soil embankments, plantings, rapid covering and re-vegetation of filled sections.
- Gas released, as a result of degradation or volatilisation of waste components, causes odour, flammability, health problems and damage of the vegetation (due to oxygen depletion in the root zone). The measures to control this include liners, soil covers, passive venting or active extraction of gas for treatment before discharge into the atmosphere.
- Polluted leachate appears shortly after disposal of the waste. This may cause groundwater pollution and pollution of streams through sub-surface migration. Liners, drainage collection, treatment of leachate, and groundwater and downstream water quality monitoring are necessary to control this problem.

3.2.9 CASE STUDY: WASTE DISPOSAL: A CASE STUDY OF BANGALORE

- One of the critical concerns of a municipal corporation is planning for a proper waste disposal in response to the increasing volume and hazardous nature of urban wastes.
- When wastes are disposed unhygienically, they do spoil the aesthetic value of the city as well as create problems such as breeding of pathogenic organisms, which serve as carriers of diseases.

- Some of the principal problems associated with disposal of solid wastes can be categorised as under:
- Diseases, i.e., rats, flies and other pests feed on the wastes and carry diseases.
- Air/noise pollution, e.g., increase in vehicular traffic, smoke, fly ash and odours.
- Ground and surface water pollution, e.g., runoff during the monsoon season causes surface water pollution, while percolation often causes groundwater contamination. Unaesthetic appearance because of litter.
- However, we can minimise or satisfactorily deal with these problems through competent engineering and planning, selecting appropriate waste disposal sites and methods of operation, and making SWM strategies essentially local.
- Against this backdrop, let us now assess the scenario in Bangalore.
- About two thirds of the waste (about 1600 tonnes/day) in the Bangalore city is getting dumped in the outskirts of the city.
- As there are no sanitary landfills in the city for proper dumping of waste, it is merely transported to the outskirts and disposed of in any abandoned open land, usually along public highways.
- The Bangalore Mahanagara Palike (BMP) along with the Karnataka State Pollution Control Board (KSPCB) has, however, identified 9 abandoned quarries around the city for sanitary landfills.

Module 4

WASTE PROCESSING TECHNIQUES & SOURCE REDUCTION, PRODUCT RECOVERY & RECYCLING:

4.1 PURPOSE OF WASTE PROCESSING

- The processing of wastes helps in achieving the best possible benefit from every functional element of the solid waste management (SWM) system and, therefore, requires proper selection of techniques and equipment for every element.
- Accordingly, the wastes that are considered suitable for further use need to be paid special attention in terms of processing, in order that we could derive maximum economical value from them.
- The purposes of processing, essentially, are

Improving efficiency of SWM system:

- Various processing techniques are available to improve the efficiency of SWM system. For example, before waste papers are reused, they are usually baled to reduce transporting and storage volume requirements.

- In some cases, wastes are baled to reduce the haul costs at disposal site, where solid wastes are compacted to use the available land effectively.
- If solid wastes are to be transported hydraulically and pneumatically, some form of shredding is also required.
- Shredding is also used to improve the efficiency of the disposal site.
- **Recovering material for reuse:**
- Usually, materials having a market, when present in wastes in sufficient quantity to justify their separation, are most amenable to recovery and recycling.
- Materials that can be recovered from solid wastes include paper, cardboard, plastic, glass, ferrous metal, aluminium and other residual metals.
- **Recovering conversion products and energy:**
- Combustible organic materials can be converted to intermediate products and ultimately to usable energy.
- This can be done either through incineration, pyrolysis, composting or bio-digestion.
- Initially, the combustible organic matter is separated from the other solid waste components.
- Once separated, further processing like shredding and drying is necessary before the waste material can be used for power generation.

4.2 MECHANICAL VOLUME AND SIZE REDUCTION:

- Mechanical volume and size reduction is an important factor in the development and operation of any SWM system.
- The main purpose is to reduce the volume (amount) and size of waste, as compared to its original form, and produce waste of uniform size.

VOLUME REDUCTION OR COMPACTION:

- Volume reduction or compaction refers to densifying wastes in order to reduce their volume.
- Some of the benefits of compaction include: reduction in the quantity of materials to be handled at the disposal site; improved efficiency of collection and disposal of wastes; increased life of landfills; Economically viable waste management system.

Equipment used for compaction:

- Based on their mobility, we can categorise the compaction equipment used in volume reduction under either of the following
- Stationary equipment: is represents the equipment in which wastes are brought to, and loaded into, either manually or mechanically.

- In fact, the compaction mechanism used to compress waste in a collection vehicle, is a stationary compactor.
- **Movable equipment:**
- This represents the wheeled and tracked equipment used to place and compact solid wastes, as in a sanitary landfill.

Location or Operation	Type of Compactor Stationary/residential	Remark
Solid waste generation points	Vertical	Vertical compaction ram may be used; may be mechanically or hydraulically operated, usually hand-fed; wastes compacted into corrugated box containers, or paper or plastic bags; used in medium and high-rise apartments
	Rotary	Ram mechanism used to compact waste into paper or plastic bags on rotating platform, platform rotates as containers are filled; used in medium and high-rise apartments.
	Bag or extrude	Compactor can be chute fed; either vertical or horizontal rams; single or continuous multi-bags; single bag must be replaced and continuous bags must be tied off and replaced; used in medium and high-rise apartments.
	Under counter	Small compactors used in individual residences and apartment units; wastes compacted into special paper bags; after wastes are dropped through a panel door into a bag and door is closed, they are sprayed for odour control; button is pushed to activate compaction mechanism.
	Stationary/commercial	Compactor with vertical and horizontal ram; wastes compressed into steel containers; compressed wastes are manually tied and removed; used in low, medium and high-rise apartments, commercial and industrial facilities.

Table 6. Types of Compaction Equipment

SIZE REDUCTION OR SHREDDING:

- This is required to convert large sized wastes (as they are collected) into smaller pieces.
- Size reduction helps in obtaining the final product in a reasonably uniform and considerably reduced size in comparison to the original form

Type	Mode of action	Application
Small grinders	Grinding, mashing	Organic residential solid wastes
Chippers	Cutting, slicing	Paper, cardboard, tree trimmings, yard waste, wood, plastics
Large grinders	Grinding, mashing	Brittle and friable materials, used mostly in industrial operation
Jaw crushers	Crushing, breaking	Large solids
Rasp mills	Shredding, tearing	Moistened solid wastes
Shredders	Shearing, tearing	All types of municipal wastes
Cutters, Clippers	Shearing, tearing	All types of municipal wastes
Hammer mills	Breaking, tearing, cutting, crushing	All types of municipal wastes, most commonly used equipment for reducing size and homogenizing composition of wastes
Hydropulper	Shearing, tearing	Ideally suited for use with pulpable wastes, including paper, wood chips. Used primarily in the papermaking industry. Also used to destroy paper records

Table 7. Types of Size reduction Equipment

4.2.1 COMPONENT SEPARATION

- Component separation is a necessary operation in which the waste components are identified and sorted either manually or mechanically to aid further processing.
- This is required for the: recovery of valuable materials for recycling; Preparation of solid wastes by removing certain components prior to incineration, energy recovery, composting and biogas production.
- The most effective way of separation is manual sorting in households prior to collection. In many cities (e.g., Bangalore, Chennai, etc., in India), such systems are now routinely used.
- The municipality generally provides separate, easily identifiable containers into which the householder deposits segregated recyclable materials such as paper, glass, metals, etc.
- Usually, separate collections are carried out for the recyclable material.
- At curbside, separate areas are set aside for each of the recyclable materials for householders to deliver material – when there is no municipal collection system.
- In case the separation is not done prior to collection, it could be sorted out through mechanical techniques such as air separation, magnetic separation, etc., to recover the wastes.

4.2.2 DRYING AND DEWATERING

- Drying and dewatering operations are used primarily for incineration systems, with or without energy recovery systems.
- These are also used for drying of sludges in wastewater treatment plants, prior to their incineration or transport to land disposal.
- The purpose of drying and dewatering operation is to remove moisture from wastes and thereby make it a better fuel.
- Sometimes, the light fraction is pelletised after drying to make the fuel easier to transport and store, prior to use in an incinerator or energy recovery facility.

4.3 SOURCE REDUCTION, PRODUCT RECOVERY AND RECYCLING

4.3.1 BASICS OF SOURCE REDUCTION

- Source reduction, also known as waste prevention, is an approach that precedes waste management and addresses how products are manufactured and, purchased.
- Put differently, this refers to the activities that reduce the amount of waste generated at source as well as activities that involve any change in the design, manufacture, purchase or usage of

materials/products to reduce their volume and/or toxicity, before they become part of the solid waste stream.

4.3.2 PURPOSE OF SOURCE REDUCTION:

Product reuse:

- Using reusable products, instead of their disposal equivalents, reduce the amount of materials that are to be managed as wastes. An example of product reuse is the reusable shopping bag.

Material volume reduction:

- Reducing the volume of material used changes the amount of waste entering the waste stream.
- This helps in controlling the waste generated and its disposal. For example, buying in bulk or using large food containers reduces the amount of packaging waste generated.

Toxicity reduction:

- Source reduction reduces the amount of toxic constituents in products entering the waste stream and reduces the adverse environmental impacts of recycling or other waste management activities.

Increased product lifetime:

- Source reduction facilitates the use of products with longer lifetime over short-lived alternatives that are designed to be discarded at the end of their useful lives.

Decreased consumption:

- This refers to the reduced consumption of materials that are not reusable.
- In brief four main advantages of source reduction are
  - Reduction in extent of environmental impacts.
  - Reduction in resource consumption and generation of pollution.
  - It includes producer, consumer, prudent and efficient activities.

4.3.3 IMPLEMENTATION OF SOURCE REDUCTION:

- **Education and research:** Consumers, businesses, industries, schools, etc., can implement education and research activities to address the need for source reduction, its consequences, available choices, benefits and costs.
- **Financial incentives and disincentives:** Linking an economic benefit to the implementation of source reduction activities encourages source reduction.
- **Regulation:** Although most regulation occurs at the national and state level, local authorities can participate in legislative activities in developing regulations that affect municipal SWM. It is possible, for example, to establish a programme to inform the consumers about environmental impacts, durability, reusability and recyclability of products as well as to declare source reduction as a top priority in SWM

4.3.4 MONITORING AND EVALUATION OF SOURCE REDUCTION

- **Monitoring**
- Monitoring facilitates the evaluation (i.e., efficacy and efficiency) of source reduction, the identification of possible source reduction measures and programme revisions and the obtaining of funds and resources for source reduction initiatives/programmes. Monitoring should, therefore, be an integral part of a source reduction programme.

  Evaluation
- Before adopting source reduction policies, it is important that we develop a framework for evaluating various options. Some of the criteria to be considered in this regard are:
- Social and economic equity.
- Economic and administrative feasibility, efficiency and cost.
- Volume requirement and scarcity of materials and natural resources in product manufacture.
- Volume of product and its by-products that must be eventually disposed.
- Useful life, reusability and/or recyclability of a product.
- Priority of source reduction of more hazardous products to less hazardous ones

4.3.5 SIGNIFICANCE OF RECYCLING

- Recycling is perhaps the most widely recognised form of source reduction involving the process of separating, collecting, processing, marketing and ultimately using a material that would have otherwise been discarded.
- This form of source reduction, i.e., recycling, is similar to other forms, in that it: lessens reliance on landfills and incinerators; protects human health and the environment by removing harmful substances from the waste stream; Conserves natural resources by reducing the demand for raw materials.
- Recycling has a lot of direct and indirect significance for the society, and this can be grouped under the following three broad areas
  - Economic significance:
  - Cost reduction
  - Employment
  - Energy saving
  - Reduced health care costs
  - Saving costs for other public utilities
  - Environmental and health significance:

- Improved environment
- Natural resource conservation
- Social significance:

- A formal recycling arrangement will help promote the social esteem of waste workers and facilitate their upward social mobility due to increased earning.
- In addition, the improved recycling activity will increase the economic value of the waste and will reduce waste scavenging activity providing opportunity for scavengers to switch to a more socially acceptable occupation.
- In short, institutionalised recycling programmes will help remove the stigma associated with waste scavenging and transform it to an economic enterprise.

4.3.6 PLANNING OF A RECYCLING PROGRAMME

- Numerous recycling options are available, and recycling programme development requires strategic planning.
- Planning for recycling involves understanding markets, assessing local expertise, setting goals and fostering public participation.
- An efficient recycling programme requires a systematic approach to all programme components, which are interrelated, and therefore, decisions about one must be made taking into consideration other components.
- The factors involved in the planning process include the following:
  - Build local expertise:
  - Understand and develop a recycling market:
  - Foster public education and involvement:
  - Assess local waste stream:
  - Augment existing programme
  - Set goals and objectives:
  - Coordinate the programme:
  - Evaluate the programme:

4.3.7 RECYCLING PROGRAMME ELEMENTS

- Recycling programmes are designed according to the needs and priorities of the communities. Elements of a recycling programme include source separation, curbside (kerbside) collection, material resource facilities and full stream processing.

- Recycling, generally, has a positive impact on other municipal waste management programmes. This may include a mix of strategies, ranging from simple, single material drop-off centres to large scale, centralised processing facilities.
- Major elements include:

Source separation:

- Source separation refers to the segregation of the recyclable and reusable materials at the point of generation

Drop-off/buy-back:

- A drop-off programme requires residents to separate the recyclable materials and bring them to a specified drop-off or collection centre.

Curbside programm:

- In a curbside system, source separated recyclables are collected separately from regular refuse from the curbside, alley, or commercial facility.

Storage and collection of recyclables:

- Collection of source-separated materials is a necessary component of recycling programme. Establishing a collection system for source-separated materials will require more careful planning than regular trash collection.
  - Collection vehicles for recycling.
  - Processing equipment for recycling'
  - Material recovery facilities (MRF).

4.3.8 COMMONLY RECYCLED MATERIALS AND PROCESSES

- **Paper and cardboard:**
- Paper recycling is one of the most profitable activities and is practised extensively. It reduces the demand for wood and energy and helps solve littering problem in the city and around dumping site.
- It has an acceptable working condition and health risks are limited.
- Recovered paper and paper products are bought and sold through a well-established network of local processors and vendors who typically bale these materials for sale.
- Glass: Glass is one of the most commonly recycled materials, and the market for post-consumer glass has historically been steady`.
- Recycling of broken glass reduces the risk of diseases caused by cuts and wounds.
- Glass recycling is a labour intensive process and provides employment opportunity.
- **Metal:** Using recycled metals substantially reduces operating costs of industries.

- Metal scrap is cheap and the energy consumption is lower when products are manufactured from scrap.
- The long-standing track record makes ferrous and non-ferrous metal market among the most stable of all recyclable materials.
- **Plastic**
- **Batteries and tyres**

4.4 CASE STUDY: SOURCE REDUCTION AND RECYCLING IN BANGALORE

- Source reduction, including reuse and recycling, can help reduce waste disposal and handling costs, because it avoids the costs of municipal composting, landfilling and combustion.
- Source reduction also conserves resources and reduces pollution, including greenhouse gases that contribute to global warming.
- Waste reduction, reuse and recycling, thus, play an important role in SWM. In what follows, we present the statistics on waste recovery and recycling done in Bangalore, India.
- In Bangalore, 66% of the waste generated is collected for recovery, i.e., about 2,373 tonnes per day. While 722 tonnes per day is reused, the rest (i.e., 1,450 tonnes) goes for recycling.
- The agents involved in the collection and recovery of wastes in the city include waste pickers, IWB (i.e., itinerant waste buyer), middlemen (or intermediaries), the municipality and recycling units (both large and small).
- While the three agents in the informal sector and the municipality are directly involved in waste collection activities, the waste is processed by the recycling units, which receive recyclable waste from middlemen and municipality.
- Of the 1450 tonnes collected for recycling, 1077.8 tonnes come from intermediaries, 60.4 come from IWB and 312 tonnes come from waste pickers. This amounts to 40% of the total waste (i.e., 3613 tonnes per day) generated.

Module 5

HAZARDOUS WASTE MANAGEMENT AND TREATMENT:

5.1 Identification and classification of hazardous waste,
5.2 Hazardous waste treatment,
5.3 Pollution prevention and waste minimization,
5.4 Hazardous wastes management in India.
5.5 E-waste recycling

5.1 IDENTIFICATION AND CLASSIFICATION OF HAZARDOUS WASTE:

- Hazardous wastes refer to wastes that may, or tend to, cause adverse health effects on the ecosystem and human beings.
- These wastes pose present or potential risks to human health or living organisms, due to the fact that they: are non-degradable or persistent in nature; can be biologically magnified; are highly toxic and even lethal at very low concentrations

5.1.1 IDENTIFICATION

- By using either or both of the following criteria, we can identify as to whether or not a waste is hazardous:
- The list provided by government agencies declaring that substance as hazardous.
- Characteristics such as ignitibility, corrosivity, reactivity and toxicity of the substance.
- Listed hazardous wastes (priority chemicals)

F-list:

- The F-list contains hazardous wastes from non-specific sources, that is, various industrial processes that may have generated the waste. The list consists of solvents commonly used in degreasing, metal treatment baths and sludges, wastewaters from metal plating operations and dioxin containing chemicals or their precursors. Examples of solvents that are F-listed hazardous wastes, along with their code numbers, include benzene (F005), carbon tetrachloride (F001), cresylic acid (F004), methyl ethyl ketone (F005), methylene chloride (F001), 1,1,1, trichloroethane (F001), toluene (F005) and trichloroethylene (F001). Solvent mixtures or blends, which contain greater

than 10% of one or more of the solvents listed in F001, F002, F003, F004 and F005 are also considered F-listed wastes.

- **K-list:**
- The K-list contains hazardous wastes generated by specific industrial processes. Examples of industries, which generate K-listed wastes include wood preservation, pigment production, chemical production, petroleum refining, iron and steel production, explosive manufacturing and pesticide production.
- P and U lists: The P and U lists contain discarded commercial chemical products, off-specification chemicals, container residues and residues from the spillage of materials. These two lists include commercial pure grades of the chemical, any technical grades of the chemical that are produced or marketed, and all formulations in which the chemical is the sole active ingredient

5.1.2 CHARACTERISTICS OF HAZARDOUS WASTES

- **Ignitability:** A waste is an ignitable hazardous waste, if it has a flash point of less than 60 C; readily catches fire and burns so vigorously as to create a hazard; or is an ignitable compressed gas or an oxidiser. A simple method of determining the flash point of a waste is to review the material safety data sheet, which can be obtained from the manufacturer or distributor of the material. Naphtha, lacquer thinner, epoxy resins, adhesives and oil based paints are all examples of ignitable hazardous wastes.
- **Corrosivity**: A liquid waste which has a pH of less than or equal to 2 or greater than or equal to 12.5 is considered to be a corrosive hazardous waste. Sodium hydroxide, a caustic solution with a high pH, is often used by many industries to clean or degrease metal parts. Hydrochloric acid, a solution with a low pH, is used by many industries to clean metal parts prior to painting. When these caustic or acid solutions are disposed of, the waste is a corrosive hazardous waste.
- **Reactivity:** A material is considered a reactive hazardous waste, if it is unstable, reacts violently with water, generates toxic gases when exposed to water or corrosive materials, or if it is capable of detonation or explosion when exposed to heat or a flame. Examples of reactive wastes would be waste gunpowder, sodium metal or wastes containing cyanides or sulphides.
- **Toxicity:** To determine if a waste is a toxic hazardous waste, a representative sample of the material must be subjected to a test conducted in a certified laboratory. The toxic characteristic identifies wastes that are likely to leach dangerous concentrations of toxic chemicals into ground water.

5.1.3 CLASSIFICATION

- **Radioactive substance:** Substances that emit ionising radiation are radioactive. Such substances are hazardous because prolonged exposure to radiation often results in damage to living organisms.
- **Chemicals:** Most hazardous chemical wastes can be classified into four groups: synthetic organics, inorganic metals, salts, acids and bases, and flammables and explosives. Some of the chemicals are hazardous because they are highly toxic to most life forms.
- **Biomedical wastes:** he principal sources of hazardous biological wastes are hospitals and biological research facilities. The ability to infect other living organisms and the ability to produce toxins are the most significant characteristics of hazardous biological wastes.
- **Flammable wastes**: Most flammable wastes are also identified as hazardous chemical wastes. This dual grouping is necessary because of the high potential hazard in storing, collecting and disposing of flammable wastes. T
- **Explosives**: Explosive hazardous wastes are mainly ordnance (artillery) materials, i.e., the wastes resulting from ordnance manufacturing and some industrial gases. Similar to flammables, these wastes also have a high potential for hazard in storage, collection and disposal
- **Household hazardous wastes**: Household wastes such as cleaning chemicals, batteries, nail polish etc. in MSW constitute hazardous waste. Especially batteries contain mercury which are alkaline which is dangerous enough to kill people

5.2 HAZARDOUS WASTE TREATMENT

- Prior to disposal, hazardous wastes need appropriate treatment, depending on the type of waste. The various options for hazardous waste treatment can be categorised under physical, chemical, thermal and biological treatments.

PHYSICAL AND CHEMICAL TREATMENT

- Physical and chemical treatments are an essential part of most hazardous waste treatment operations, and the treatments include the following
- **Filtration and separation:** Filtration is a method for separating solid particles from a liquid using a porous medium. The driving force in filtration is a pressure gradient, caused by gravity, centrifugal force, vacuum, or pressure greater than atmospheric pressure
- **Che**mical precipitation: This is a process by which the soluble substance is converted to an insoluble form either by a chemical reaction or by change in the composition of the solvent to diminish the solubility of the substance in it. Settling and/or filtration can then remove the precipitated solids. In the treatment of hazardous waste, the process has a wide applicability in the removal of toxic metal from aqueous wastes by converting them to an insoluble form.

- **Chemical oxidation and reduction (redox):** In these reactions, the oxidation state of one reactant is raised, while that of the other reactant is lowered. When electrons are removed from an ion, atom, or molecule, the substance is oxidised and when electrons are added to a substance, it is reduced. Such reactions are used in treatment of metal-bearing wastes, sulphides, cyanides and chromium and in the treatment of many organic wastes such as phenols, pesticides and sulphur containing compounds.
- **Solidification and stabilisation**: In hazardous waste management, solidification and stabilisation (S/S) is a term normally used to designate a technology employing activities to reduce the mobility of pollutants, thereby making the waste acceptable under current land disposal requirements.
- **Evaporation:** Evaporation is defined as the conversion of a liquid from a solution or slurry into vapour. All evaporation systems require the transfer of sufficient heat from a heating medium to the process fluid to vaporise the volatile solvent. Evaporation is used in the treatment of hazardous waste and the process equipment is quite flexible and can handle waste in various forms – aqueous, slurries, sludges and tars.
- **Ozonation:** Ozone is a relatively unstable gas consisting of three oxygen atoms per molecule (O3) and is one of the strongest oxidising agents known. It can be substituted for conventional oxidants such as chlorine, hydrogen peroxide and potassium permanganate. Ozone and UV radiations have been used to detoxify industrial organic wastes, containing aromatic and aliphatic polychlorinated compounds, ketones and alcohols.

THERMAL TREATMENT

- **Incineration:** Incineration can be regarded as either a pre-treatment of hazardous waste, prior to final disposal or as a means of valorising waste by recovering energy. It includes both the burning of mixed solid waste or burning of selected parts of the waste stream as a fuel.
- **Pyrolysis:** This is defined as the chemical decomposition or change brought about by heating in the absence of oxygen. This is a thermal process for transformation of solid and liquid carbonaceous materials into gaseous components and the solid residue containing fixed carbon and ash.

BIOLOGICAL TREATMENT

- **Land treatment:** This is a waste treatment and disposal process, where a waste is mixed with or incorporated into the surface soil and is degraded, transformed or immobilised through proper management. The other terminologies used commonly include land cultivation, land farming, land application and sludge spreading.

- **Enzymatic systems**: Enzymes are complex proteins ubiquitous in nature. These proteins, composed of amino acids, are linked together via peptide bonds. Enzymes capable of transforming hazardous waste chemicals to non-toxic products can be harvested from microorganisms grown in mass culture. Such crude enzyme extracts derived from microorganisms have been shown to convert pesticides into less toxic and persistent products.
- **Composting:** The principle involved in composting organic hazardous wastes are the same as those in the composting of all organic materials though with moderate modifications. The microbiology of hazardous wastes differs from that of composting in the use of inoculums.
- Aerobic and anaerobic treatment: Hazardous materials are present in low to high concentration in wastewaters, leachate and soil. These wastes are characterised by high organic content (e.g., up to 40,000 mg/l total organic carbon), low and high pH (2 to 12), elevated salt levels (sometimes, over 5%), and presence of heavy metals and hazardous organics. Hazardous wastes can be treated using either aerobic or anaerobic treatment methods.

5.3 POLLUTION PREVENTION AND WASTE MINIMISATION

- Pollution prevention is the use of materials, processes, or practices that reduce or eliminate the generation of pollutants or wastes at the source.
- It includes practices that reduce the use of hazardous and non-hazardous materials, energy, water or other resources as well as those that protect natural resources through conservation or more efficient use.
- Pollution prevention is the maximum feasible reduction of all wastes generated at production sites.
- It involves the judicious use of resources through source reduction, energy efficiency, and reuse of input materials and reduces water consumption.

  Factors that can contribute to pollution prevention and waste minimisation.
- **Management support and employee participation:** A clear commitment by management (through policy, communications and resources) for waste minimisation and pollution prevention is essential to earn the dedication of all employees.
- For this to happen, a formal policy statement must be drafted and adopted.
- The purpose of this statement is to reflect commitment and attitude towards protecting the environment, minimising or eliminating waste and reusing or recycling materials by the laboratories, departments and industries.
- Creative, progressive and responsible leadership will serve to develop an environmental policy.
- However, the total employee workforce will need to be involved to realise the fruits of the planning.

- **Training:** As with any activity, it is important for management to train employees so that they will have an understanding of what is expected of them and why they are being asked to change the way things are done.
- Employees must be provided with formal and on-the-job training to increase awareness of operating practices that reduce both solid and hazardous waste generation.
- The training programme should include the industries' compliance requirements, which may be found in the waste management policies, occupational health and safety requirements.
- Additionally, training on waste minimisation and pollution prevention is necessary.
- **Waste audits:** A programme of waste audits at the departmental level will provide a systematic and periodic survey of the industries designed to identify areas of potential waste reduction.
- The audit programme includes the identification of hazardous wastes and their sources, prioritisation of various waste reduction actions to be undertaken, evaluation of some technically, economically and ecologically feasible approaches to waste minimisation and pollution prevention, development of an economic comparison of waste minimisation and pollution prevention options and evaluation of their results.
- **Good operating practices:** These practices involve the procedural or organisational aspects of industry, research or teaching activities and, in some areas, changes in operating practices, in order to reduce the amount of waste generated.
- These practices would include, at a minimum, material handling improvements, scheduling improvements, spill and leak prevention, preventive maintenance, corrective maintenance, material/waste tracking or inventory control and waste stream segregation, according to the toxicity, type of contaminant and physical state.
- **Material substitution practices:** The purpose of these practices is to find substitute materials, which are less hazardous than those currently utilised and which result in the generation of waste in smaller quantities and/or of less toxicity.
- **Technological modification practices:** These practices should be oriented towards process and equipment modifications to reduce waste generation. These can range from changes that can be implemented in a matter of days at low cost to the replacement of process equipment involving large capital expenditures.
- **Recycling options:** These options are characterised as use/reuse and resource recovery techniques. Use and reuse practices involve the return of a waste material either to the originating process or to another process as a substitute for an input material. Reclamation practices tender a waste to another company.

- **Surplus chemical waste exchange options:** Inter- and intra-department chemical exchange is to be implemented and encouraged by employers/employees. Material exchanges not only reduce wastes but also save money – both are important considerations, during times of fiscal crisis.

5.4 HAZARDOUS WASTES MANAGEMENT IN INDIA

- In the USA, more than 70% of the hazardous waste generated was produced from chemical and petrochemical industries.
- Of the remaining waste produced, 22% was generated by metal related industries. As industrialisation proceeds, the management of hazardous wastes is increasingly becoming a serious problem in India as well.
- The Indian chemical industry, which accounts for about 13% of the total industrial production and about 10% of the GNP valued at US $ 2.64 X 1011 (NNP is US $ 2.345 X 1011) per annum, employs about 6% of the nation's industrial workforce and is one of the major generators of toxic and hazardous wastes.
- There are 13,011 industrial units located in 340 districts, out of which 11,038 units have been granted authorization for multiple disposal practices encompassing incineration, storage land disposal and other disposal options.
- However, small and medium sized enterprises (SMEs) are the major sources of hazardous wastes. And, the States of Andhra Pradesh, Assam, Gujarat, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Orissa, Rajasthan and Tamil Nadu generate the majority of all hazardous wastes.
- The total estimate of hazardous waste generated in India is 4,434,257 tonnes per annum.
- India is the first country that has made provisions for the protection and improvement of environment in its Constitution.
- The Directive Principles of State Policy of the Constitution, Article 48-A of Chapter IV enjoins the State to make endeavour for protection and improvement of the environment and for safeguarding the forest and wild life of the country.
- In Article 51 A (g) of the Constitution, one of the Fundamental Duties of every citizen of India is to protect and improve the natural environment including forests, lakes, rivers and wild life and to have compassion for living creatures. India has enacted the following laws, regulations and standards governing the country's environmental protection:
- The Water (Prevention and Control of Pollution) Act, 1974 as amended in 1988.
- Water (Prevention and Control of Pollution) Rules, 1975.
- The Water (Prevention and Control of Pollution) Cess Act, 1977, as amended by Amendment Act, 1991.

- The Water (Prevention and Control of Pollution) Cess Rules, 1978.
- The Air (Prevention and Control of Pollution) Act, 1984, as amended by Amendment Act, 1987.
- The Air (Prevention and Control of Pollution) Rules 1982 and 1983. (vii) The Environment (Protection) Act, 1986
- Hazardous Waste (Management and Handling) Rules, 1989 as amended in 2000.
- Management, Storage and Import of Hazardous Chemical Rules, 1989.
- Manufacture, Use, Import, Export and Storage of Hazardous Microorganisms, Genetically Engineered Microorganisms or Cells Rules, 1989.
- The Public Liability Insurance Act, 1991. (xii) The Public Liability Insurance Rules, 1991.
- The Biomedical Wastes (Management and Handling) Rules, 1995.
- Municipal Wastes (Management and Handling) Draft Rules, 1999.
- Hazardous Waste (Management and Handling) Amendment Rules 2000

5.5 E-WASTE RECYCLING PROCESS

- The amount of e-waste generated around the world in recent years has exploded, driven by changes in technology, planned obsolescence, changes in media and storage types (tapes, CDs, HDs, SSDs etc.), and easier accessibility through decreasing costs.
- As the availability and use of electronics increases across the globe, e-waste has become the fastest-growing waste stream in the world.
- E-waste refers to any electronic devices that have reached the end of life. Unfortunately, many of the items that are labeled as "e-waste" are in fact not, since old devices that are no longer wanted but still working (or suitable for repair) can be donated, reused, or refurbished.

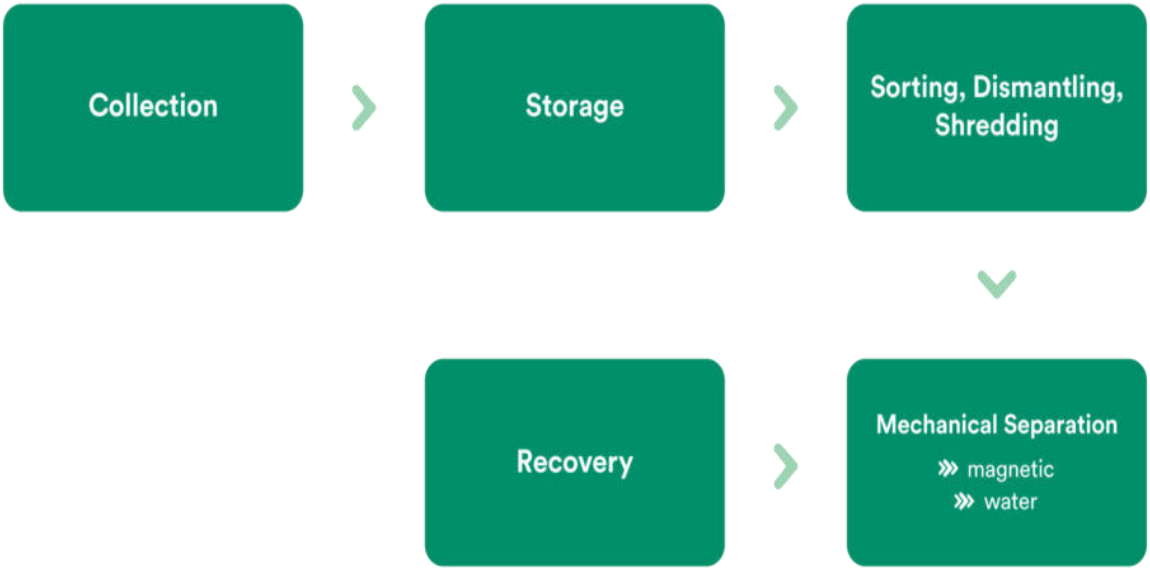

Fig 7 E-waste recycling process flowchart

- The e-waste recycling process

Step One — Collection

- The first stage in the recycling process for e-waste is the collection of electronic products through recycling bins, collection locations, take-back programs, or on-demand collection services. The mixed e-waste is then taken to specialized electronics recyclers.
- Best practice dictates that e-waste should be separated by type at this stage of the process, which is why many collection sites will have different bins or boxes for different items. This is especially important for e-waste containing batteries, which require special treatment and can be very damaging if mixed with other waste.

Step Two — Storage

- While safe storage may not appear critical, it can prove very important. For example, the glass screens of Cathode Ray Tubes (CRT) TVs and monitors are highly contaminated by lead. In the past, they were recycled into new computer monitors, but the growth of new technology and subsequent decline in demand for CRT products means much of this glass is now simply being stored indefinitely.

Step Three — Manual Sorting, Dismantling, Shredding

- E-waste then goes through the initial stage of manual sorting, where various items (such as batteries and bulbs) are removed for their own processing. This is the stage at which some items may also be manually dismantled for components, reuse, or the recovery of valuable materials.
- E-waste is then shredded into small pieces allowing for accurate sorting of materials, a key part of the process. Most electronics are a mix of materials, and breaking items down into pieces that measure just a few centimeters means they can be separated mechanically.

Step Four — Mechanical Separation

- The mechanical separation of the different materials actually consists of several processes one after the other. The two key steps are magnetic separation and water separation

Magnetic Separation

- The shredded e-waste is passed under a giant magnet, which is able to pull ferrous metals such as iron and steel from the mix of waste.
- In addition to this, an eddy current may also be used, separating the nonferrous metals.
- These materials can then be diverted to dedicated recycling plants for smelting.
- Other materials such as metal-embedded plastic and circuit boards are also separated at this stage.

Water Separation

- With a solid waste stream that now consists mainly of plastic and glass, water is used to separate the materials, further purifying for the separation of different plastics as well as hand-sorting

obvious contaminants.

Step Five: Recovery

- The materials, now separated, are prepared for sale and reuse. For some materials, such as plastic or steel, this means joining another recycling stream. Others may be processed onsite and sold directly alongside usable components separated in the early stages.
- How the universal recycling process differs across common items
- While this represents the general e-waste management recycling process, many items have their own unique processes. For example:

The Recycling Process for Batteries

- Upon arrival at a site, batteries are sorted by chemistry—lead-acid, nickel-cadmium, nickel-metal-hydride, and lithium-ion. Combustible materials, such as plastic casings and insulation, are burned off, with a scrubber being used to capture polluting particles and gasses created during the incineration process.
- The emptied metal cells are then chopped into pieces and heated until the metal liquefies, and non-metal components burn and gather on the top as a substance known as slag, which is scraped from the surface.
- At this point, some centers send unprocessed metal to specialized recycling plants. Other plants collect the metals during the liquification process since they settle in layers according to density. Cadmium vaporizes during this process and is collected through a condensation process

The Recycling Process for Cathode Ray Tubes

- Cathode Ray Tubes are considered one of the most troublesome types of waste to recycle. While many of their components can be broken down, they can contain as much as four pounds of lead per monitor/TV.
- This represents a significant threat, and the glass is so contaminated by this lead that it can't be added to normal glass recycling streams. While this outdated technology might not seem like a problem going forward, there remains a huge issue regarding recycling old items.

The E-waste Recycling Process for Computers and Laptops

- The process for recycling laptops and computers is very similar to the general process outlined above. However, there is likely to be a greater focus on manual sorting and separation since computer components from broken machines can be combined into new computers with no extra resources.
- What's more, the e-waste recycling process is also likely to include some sort of data destruction. This will be carried out digitally by wiping those hard drives that are reusable, or physically, by

shredding them or using other data destruction methods. Businesses and individuals are increasingly concerned about data protection, and the shredding of confidential paper documents is already common practice. Destroying data on hard drives is simply the 21st-century equivalent.

- Finally, the Basel Convention identified e-waste as a problem back in 2002, and yet, we are only just at the beginning of a long journey towards an ideal zero e-waste world. Our digital world is here to stay, and if we continue to consume non-recyclable equipment at current rates without comprehensive reduction, reuse, and recycling programs in place, we will quickly deplete natural resources and create e-scrap on an unimaginable scale.
- However, there is hope that we can strive for a more circular economy with e-waste, and both businesses and individuals can make a difference through conscientious consumption, reduction, and reuse, eventually pushing manufacturers towards more easily recyclable devices through producer responsibility programs.

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Hand Book on

Emerging Applications of Biosensors

BETC152

For First Year BE, VTU, Belgaum

Course Title:	**Emerging applications of biosensors**		
Course Code:	BETC152	CIE Marks	50
Course Type (Theory/Practical /Integrated)	Theory	SEE Marks	50
		Total Marks	100
Teaching Hours/Week (L:T:P: S)	3:0:0:0	Exam Hours	3 hrs of Theory
Total Hours of Pedagogy	40 hours	Credits	03

Course objectives

To learn the Fundamentals of biosensors.

To acquaint the student with design and construction of biosensors.

To expose the students to recent advances in application of biosensors in health, environment, agriculture and food industry.

Module-1 (8)

INTRODUCTION TO BIOSENSORS

Introduction to biosensor, General components of biosensor, Biomolecules in biosensors such as enzyme, DNA, antigen antibody, protein, Classification of biosensors based on principle: amperometric, potentiometric biosensors, optical, acoustic, piezoelectric, and calorimetric biosensors, scope of biosensors and its limitations.

Module-2 (8)

BASIC DESIGN AND TRANSDUCER

Design Considerations: calibration, dynamic Range, signal to noise, sensitivity, selectivity, Interference recognition. Transduction membrane protein sensors: ion channels, Types of Transducer, Optical; Fiber Optic, ECL, Surface Plasmon Resonance, Electro chemical; FET, Impedance, Piezoelectric; Cantileaver,

Module-3(8)

APPLICATIONS OF BIOSENSORS IN HEALTH AND ENVIRONMENT

Biosensors and diabetes management, Microfabricated biosensors and point-of-care diagnostics systems, Noninvasive biosensors in clinical analysis; Surface plasmon resonance and evanescent wave biosensors, Biosensor in cancer and HIV early diagnosis.

Module-4(8)

APPLICATIONS OF BIOSENSORS IN FOOD AND AGRICULTURE INDUSTRY

Detection of product content, allergic components, pathogens, pesticide residues. Monitoring of raw material conversions. Detection of crop diseases, pathogens in plants, Detection of soil nutrients, pesticide and its residual detection.

Module-5 (8)

APPLICATIONS OF NANOMATERIALS IN BIOSENSORS

Nano Materials in biosensors; Carbon based Nano Material, Metal oxide and nano particle, Quantum dots, Role of nano material in Signal Amplifications, Detection and Transducer Fabrication

Course outcome (Course Skill Set)

At the end of the course the student will be able to:

CO1	Classify types of biosensors based on principle
CO2	Able to differentiate different types of transducers based on their physicochemical characteristics
CO3	Apply bio sensing techniques in health, environment, and agriculture and food industry.
CO4	Use biomaterial and nanomaterials in biosensors for signal amplification, Detection and Transducer Fabrication

Module 1

INTRODUCTION TO BIOSENSORS

1.1 Introduction to biosensor,

1.2 General components of biosensor,

1.3 Biomolecules in biosensors such as enzyme, DNA, antigen antibody, protein,

1.4 Classification of biosensors based on principle: amperometric, potentiometric biosensors, optical, acoustic, piezoelectric, and calorimetric biosensors,

1.5 Scope of biosensors and its limitations.

1.1 Introduction to Biosensor

- Sensor is a device which detects changes in a physical quantity like temperature, humidity, water flow, intensity of light etc. and converts it into a quantity that can be measured and/or analysed.
- Biosensor is an analytical device that converts a biological response into a more useful electrical signal using biological responsive material or bio recognition element such as microorganisms, cells, enzyme, antibody or nucleic acid.
- Biosensor is a combination of a Biological sensing element and a transducer, which converts the data into electrical signals. Additionally, there will be an electronic circuit which consists of a Signal Conditioning Unit, a Processor or Microcontroller and a Display Unit.
- Biosensors are self-sufficient integrated devices that has capacity to provide specific qualitative or semi-quantitative analytical information using a biological recognition element which is in direct-spatial contact with a transductional element.
- In simple words, biosensors are analytical devices that detects changes in biological processes and transform the biological data into electrical signal.
- The main features of biosensors are, Stability, Economical, Sensitivity and Reproducibility.
- Biorecognition elements should be highly specific for the analyte.
- Biorecognition elements should be stable under assay conditions over a very large number of assays
- The reaction should be independent of physical parameters such as stirring, pH and temperature
- Response should accurate, precise, reproducible, and linear over useful analytical range.
- Invasive biosensors should be tiny, biocompatible and sterelizable, if required.
- Complete biosensor should be cheap, small, portable and easy to operate.
- The desired biological material is usually in the form of an enzyme due to operate.

- One of the commonly used biorecognition element is enzyme due to its advantages such as specificity, rate enhancement, regulation, reuse, stability, immobilisation etc.
- General components of biosensors are bio recognition element, transducer, amplifier, signal processing unit and display unit.
- Biosensors have become very important in the fields of medicine, clinical analysis and in general health monitoring.
- The advantages of biosensors over lab based equipment are their small size, low cost, quick results, and very easy to use.
- Biosensors are used in Medicine, Clinical and Diagnostic Applications, Environmental Monitoring, Industrial Applications, Food Industry and Agriculture Industry
- Apart from the desired medicine and health based applications, Biosensors have also found critical applications in several other fields like industrial processing, agriculture, food processing, pollution control etc.
- Commercial Biosensor in the field of personal health care are becoming quite popular, especially, self-monitoring of blood glucose.

1.2 General components of biosensor

- Generally, biosensors are composed of three main components as depicted in Figure 1.
- These include a biological sensing element, transducer, and signal processing unit.
- The block diagram of the biosensor consists of three segments namely, biological sensing element, transducer, and signal processing unit**.**

1.2.1 Biological sensing element:

- This component is also known as a sensor or detector element and is responsible for sensing or detecting the presence and/or the concentration of the target analyte or substance.
- This is a biological component, which serves as a biochemical receptor that specifically recognizes the target analyte.
- When the biological receptor interacts with a target analyte, it generates a signal in the form of light, heat, pH, charge or mass change.
- This material should be highly specific, stable under storage conditions and must be immobilized.
- Furthermore, the biological receptor should be capable of selectively detecting the target compound or analyte in the test sample.
- Biological receptor determines the sensitivity of the entire device through the generation of the physicochemical signal that is monitored by the transducer.

- This component can be a tissue, microorganism, organelle, cell receptor, enzyme, antibody or nucleic acid etc. These can be grouped into two categories, namely catalytic and non-catalytic receptors.
- The catalytic group of biological receptors are used in devices intended for continuous monitoring of substances at millimolar or micromolar concentrations.
- These include enzymes, tissues and microorganisms.
- The non-catalytic group is used mainly in biosensor devices that measure analytes such as steroids, drugs, and toxins etc. which usually occur at very low concentrations (micro to picomollar range)
- These are non-reusable devices which can only be used once and discarded thereafter. Such receptors include antibodies, antigens, nucleic acids etc.

1.2.2 Transducer:

- The second segment of the biosensor is the transducer and it is a physical component.
- Transducer converts the biochemical signal into proportional electrical signals.
- Generally, a transducer is a material that is capable of converting one form of energy to another.
- In a biosensor, a transducer is responsible for converting the biochemical signal received from the biological receptor, which is a result of the interaction between the target analyte and the biological receptor, into a measurable and quantifiable signal which can be piezo-electrical, optical, electrochemical etc.
- The transducer detects and measures the change that occurs during biological receptor – analyte interaction.
- An example of a transducer is a pH sensor in a glucose biosensor.
- An enzyme, known as glucose oxidase, is used as a biological receptor which binds glucose and converts it to gluconic acid in the presence of oxygen.
- The pH sensor (transducer) then detects the change in pH (due to production of gluconic acid) and converts it into a voltage change.
- The following features are recommended when a transducer is designed; specificity to the target analyte, analyte concentration range, response time and suitability for practical applications.
- Ideally, a transducer should be highly specific to the analyte, give measurement at the lowest analyte concentration within the shortest time possible.

1.2.3 Signal processing Unit:

- The output of the transducer will be either current or voltage relying on the type of enzyme.
- If the output is voltage, then it is fine.

- But if the output is current, then this current needs to be converted into equivalent voltage (using an Op-Amp based current to voltage converter) before proceeding further.
- The output voltage signal is generally very low in amplitude and is superimposed on a high frequency noise signal.
- Thus, the signal is amplified (using an Op-Amp based Amplifier) and then it is passed through a Low Pass RC Filter.
- Signal Processing Unit or a Signal Conditioning Unit is accountable for performing the process of amplifying and filtering the signal. The output of the signal processing unit is termed as an analog signal.
- This output is equivalent to the biological quantity being measured.
- The analog signal can be exhibited directly on an LCD display but usually, this analog signal is passed to a Microcontroller, where the analog signal is converted into digital signal.
- This is done since it is easy to analyse, process or store a digital signal.
- Signal processing unit is the associated part which consists of Signal Conditioning Unit, a Processor or Micro-controller and a Display Unit.

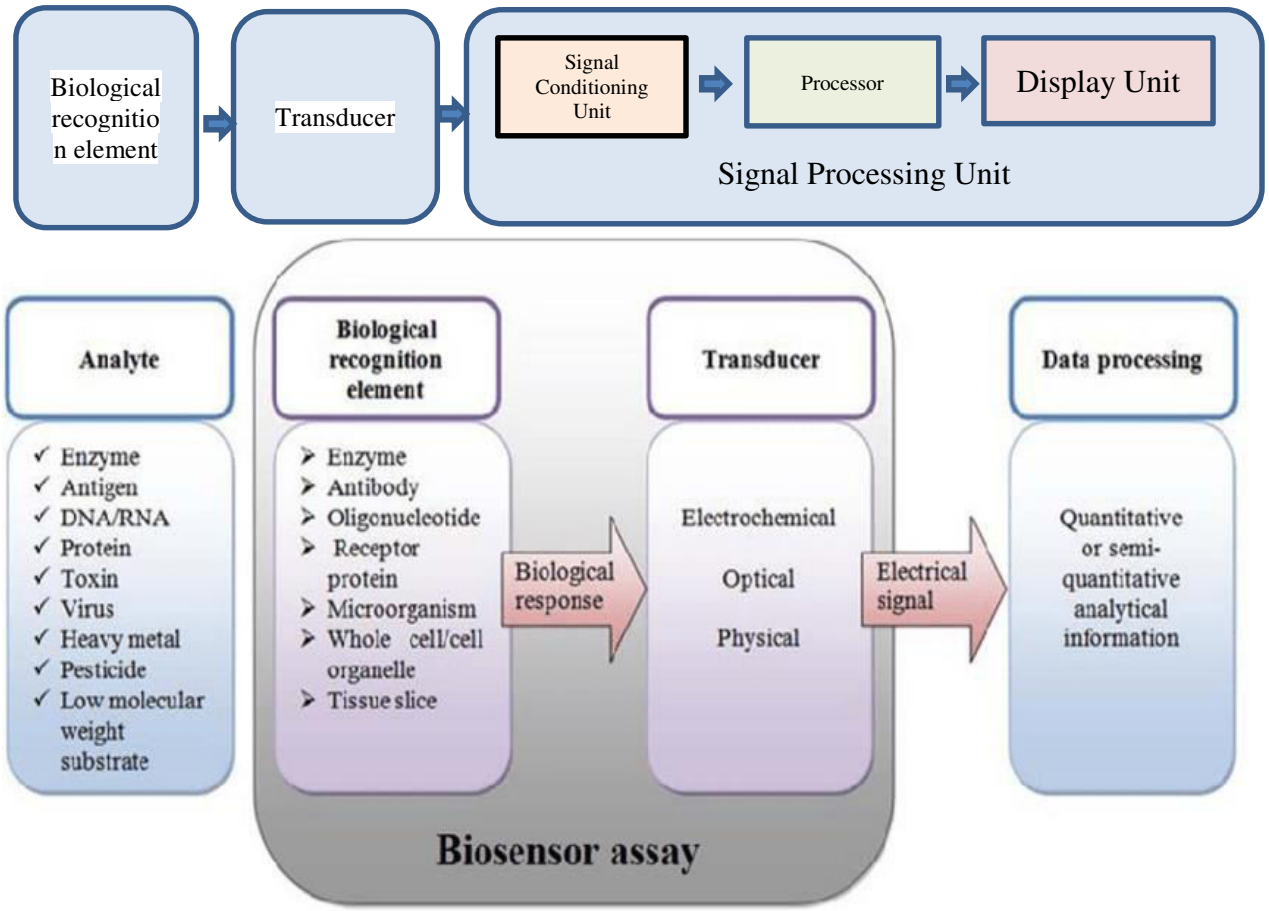


Fig 1.Generalised representation of Biosensors

1.3 Biomolecules in biosensors such as enzyme, DNA, Antigen-Antibody, Protein.

1.3.1 Enzyme based biosensors

- Enzyme based biosensors are most popular biosensors due to its specialist features such as specificity, rate enhancement, regulation and mild working condition.
- Enzyme biosensors are useful tools for monitoring rapid changes in metabolite levels in real-time, include pure enzyme preparations or biological processes.
- They have been derived on immobilization processes such as van der Waals forces, ionic or covalent bonding.
- The well-known enzymatic biosensors today are glucose and urea biosensors.
- However, glucose biosensors are most popular among researchers and are reportedly the mostly commercialized biosensors.
- The glucose biosensor, which was developed by Clark, is made up of glucose oxidase immobilized within a dialysis membrane which is integrated inside oxygen electrodes.
- Enzymatic biosensors are known for their prolonged use and reusability due to the fact that enzymes used as biological receptors cannot be consumed.
- Thus, the detection limit and the lifetime of enzyme based biosensors is greatly enhanced by the stability of the enzyme.

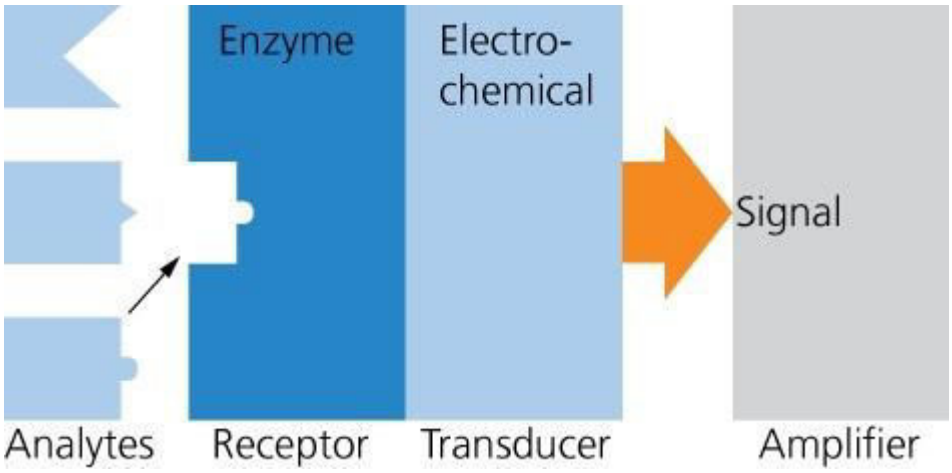


Fig 2 Enzyme based biosensors

1.3.2 DNA based biosensors

- Another group of biosensors based on a biological receptor is DNA biosensors.
- The most attractive feature of biosensors is the high selectivity of biosensors for their target analytes in a matrix of chemical or biological elements.

- DNA biosensors, which use nucleic acids as their biological receptors, detect proteins and non-macromolecular compounds that interact with certain DNA fragments known as DNA probes or DNA primers.
- The interaction observed stems from the formation of stable hydrogen bonds between the double helix nucleic acid strands.
- To develop DNA biosensors, immobilization of the probe becomes the most crucial step.
- The strong pairing of lined up nucleotide strands between bases in their complementary parts influences biosensors based on DNA, RNA, and peptide nucleotide acids to be the most sensitive tool.
- That probes, which are short oligonucleotides capable of hybridization with individual areas of the target nucleotide sequence, together with various chemical composition and conformational arrangements, were employed in the development of DNA biosensors.
- Extremely high sensibility and selectivity is needed to maximize the hybridization efficiency and minimize non-specific binding.

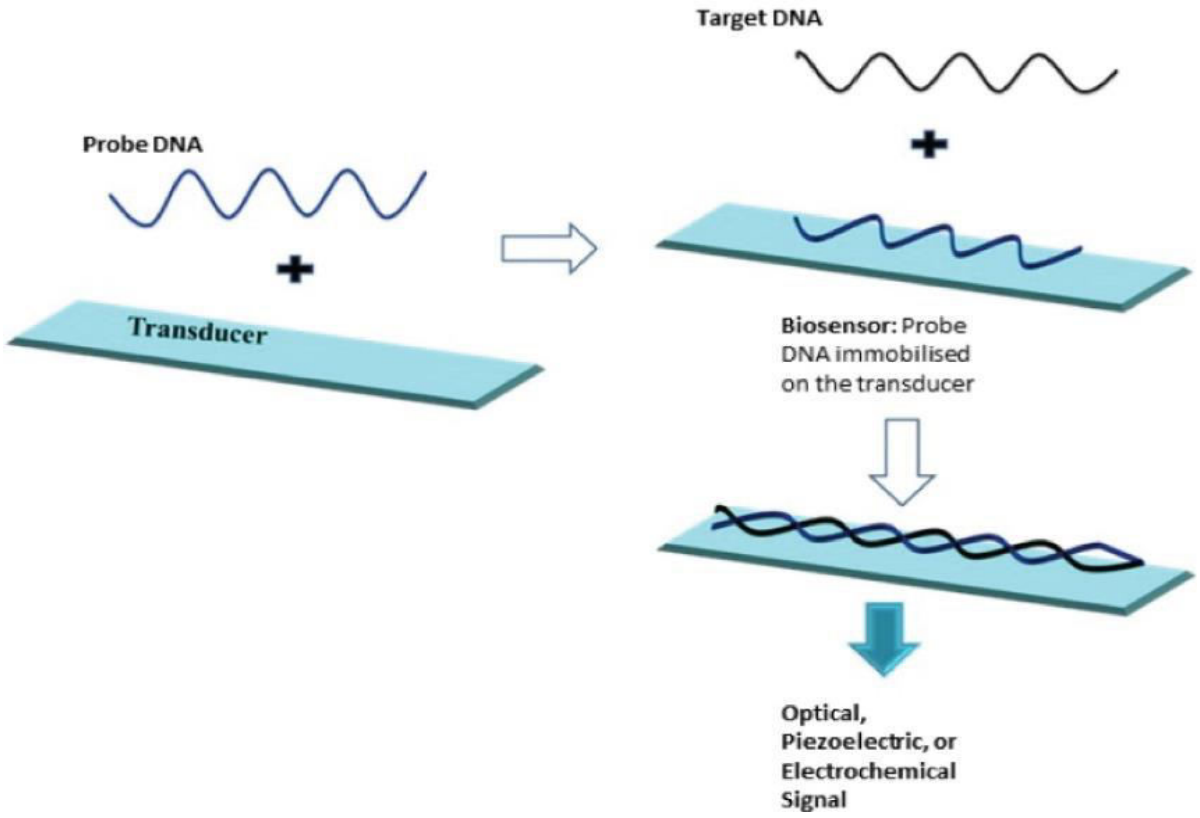


Fig 3 DNA based biosensors

1.3.3 Antibody based sensors

- Antibody-based biosensors or immunosensors have revolutionized diagnostics for the detection of a plethora of analytes such as disease markers, food and environmental contaminants, biological warfare agents and illicit drugs.

- Antibodies are ideal biorecognition elements that provide sensors with high specificity and sensitivity.
- Antibodies are large Y-shaped proteins produced by plasma cells that are utilized by the immune system to identify and target pathogens such as bacteria and viruses.
- Their small size, high stability and easy genetic manipulation make recombinant antibody fragments valuable and robust tools for the fabrication of immunosensors.
- Antibody-based biosensors have revolutionized diagnostics for the detection of a plethora of analytes such as food and environmental contaminants, biological warfare agents, illicit drugs and disease markers.
- Immobilization of antibodies on to a sensor surface without altering their specificity and immunological activity is one of the most crucial steps in the fabrication of a successful immunosensor.
- The immobilization step affects the detection limit, sensitivity and overall performance of the immunosensor.
- Orientation of antibodies on sensor surfaces can be controlled by the interaction between specific reactive groups on the surface and on the antibody.

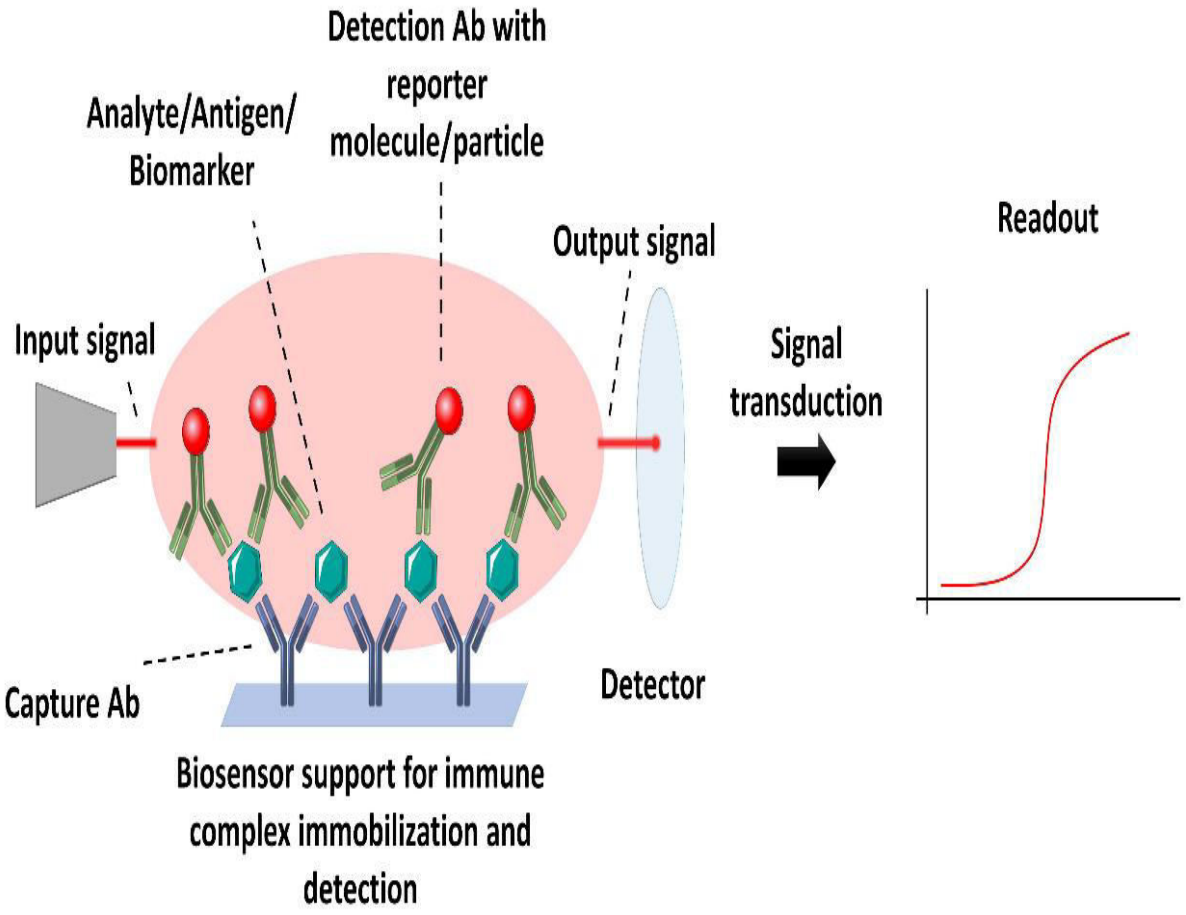


Fig 4 Antibody based biosensors

1.3.4 Protein based sensors

- Peptides/peptides have been used as components in biological analysis and fabrication of novel biosensors for a number of reasons, including mature synthesis protocols, diverse structures and as highly selective substrates for enzymes.
- Bio-conjugation strategies can provide an efficient way to convert interaction information between peptides and analytes into a measurable signal, which can be used for fabrication of novel peptide/protein-based biosensors.
- Many sensitive fluorophores can respond rapidly to environmental changes and stimuli manifest as a change in spectral characteristics, hence environmentally-sensitive fluorophores have been widely used as signal markers to conjugate to peptides to construct peptide-based molecular sensors.
- Additionally, nanoparticles, fluorescent polymers, graphene and near infrared dyes are also used as peptide-conjugated signal markers.
- On the other hand, peptides/proteins may play a generalist role in peptide-based biosensors.
- Peptides/proteins have been utilized as biorecognition elements to bind various analytes including proteins, nucleic acid, bacteria, metal ions, enzymes and antibodies in biosensors.
- The selectivity of peptides/proteins as an enzymatic substrate has thus been utilized to construct enzyme sensors or enzyme-activity sensors.
- In addition, progress on immobilization and microarray techniques of peptides has facilitated the progress and commercial application of chip-based peptide biosensors in clinical diagnosis.

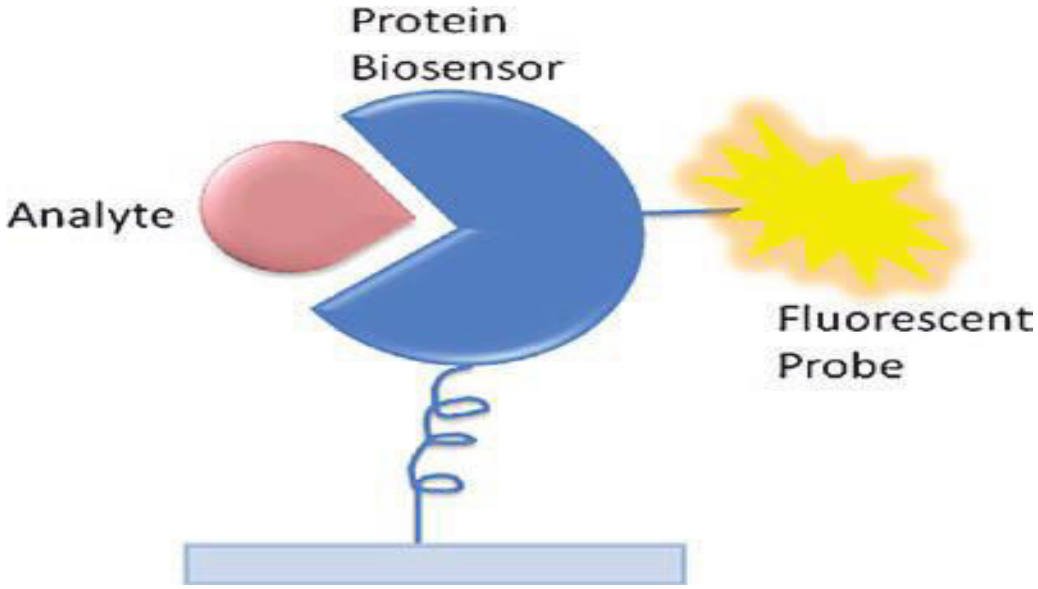


Fig 5 Antibody based biosensors

1.4 Classification of biosensors based on principle: Amperometric, Potentiometric, Optical, Acoustic, Piezoelectric, and Calorimetric Biosensors

- The most commonly applied classification of biosensors is based on the type of transduction element used in the sensor.
- These biosensors are grouped into three main categories, amperometric, potentiometric, optical, acoustic, piezoelectric, and calorimetric biosensors.
- The working principles of each of the three biosensors are different and can thus be implemented in a variety of applications.

Based on the working principles of Transducer of the biosensor, classification is done as follows

- **Amperometric Biosensor:** Based on the movement of electrons produced in the redox reaction.
- **Potentiometric Biosensor:** Based on the changes in the distribution of charge causing an electrical potential to be produced.
- **Optical Biosensor:** Light output during the reaction or light absorption difference between the reactants and products.
- **Acoustic Biosensor:** Utilizes acoustic or mechanical waves as a detection mechanism to obtain medical, biochemical, and biophysical information about the analyte of interest
- **Piezoelectric Biosensor:** Piezoelectric effect due to the mass of the reactants
- **Calorimetric Biosensors:** Heat output input by the reaction

1.4.1 Amperometric biosensors

- Amperometric biosensors function by the production of a current when a potential is applied between two electrodes.
- The simplest amperometric biosensors in common usage involve the Clark oxygen electrode.
- This consists of a platinum cathode at which oxygen is reduced and a silver/silver chloride reference electrode.
- When a potential of -0.6 V, relative to the Ag/AgCl electrode is applied to the platinum cathode, a current proportional to the oxygen concentration is produced.
- Normally both electrodes are bathed in a solution of saturated potassium chloride and separated from the bulk solution by an oxygen-permeable plastic membrane (e.g., Teflon, polytetrafluoroethylene).
- The following reactions occur:

Ag anode

$$\left\{\begin{array}{l} 4Ag \longrightarrow {}^{+} + 4e^{-} \\ 4Ag^{+} + 4Cl^{-} \longrightarrow 4AgCl \end{array}\right\}$$

$$4Ag^{+} + 4Cl^{-} \longrightarrow 4AgCl + 4e^{-}$$

Pt cathode

$$\left\{\begin{array}{l} O_2 + 2H_2O + 2e^{-} \longrightarrow 2H_2O + 2OH^{-} \\ 2H_2O + 2e^{-} \longrightarrow 2OH^{-} \end{array}\right\}$$

$$O_2 + 4H^{+} + 4e^{-} \longrightarrow 2H_2O$$

- The efficient reduction of oxygen at the surface of the cathode causes the oxygen concentration there to be effectively zero.
- The rate of this electrochemical reduction therefore depends on the rate of diffusion of the oxygen from the bulk solution, which is dependent on the concentration gradient and hence the bulk oxygen concentration.
- It is clear that a small, but significant, proportion of the oxygen present in the bulk is consumed by this process; the oxygen electrode measuring the rate of a process which is far from equilibrium, whereas ion-selective electrodes are used close to equilibrium conditions.
- This causes the oxygen electrode to be much more sensitive to changes in the temperature than potentiometric sensors.
- A typical application for this simple type of biosensor is the determination of glucose concentrations by the use of an immobilised glucose oxidase membrane.
- The reaction results in a reduction of the oxygen concentration as it diffuses through the biocatalytic membrane to the cathode, this being detected by a reduction in the current between the electrodes.
- Other oxidases may be used in a similar manner for the analysis of their substrates (e.g., alcohol oxidase, D- and L-amino acid oxidases, cholesterol oxidase, galactose oxidase, and urate oxidase).

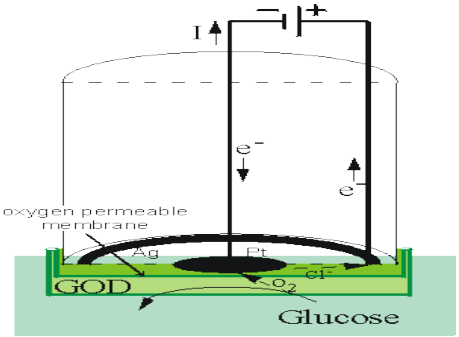


Figure 6. Schematic diagram of a simple amperometric biosensor. A potential is applied between the central platinum cathode and the annular silver anode.

- This generates a current (I) which is carried between the electrodes by means of a saturated solution of KCl.
- This electrode compartment is separated from the biocatalyst (here shown glucose oxidase, GOD) by a thin plastic membrane, permeable only to oxygen. T
- he analyte solution is separated from the biocatalyst by another membrane, permeable to the substrate(s) and product(s).
- This biosensor is normally about 1 cm in diameter but has been scaled down to 0.25 mm diameter using a Pt wire cathode within a silver plated steel needle anode and utilising dip-coated membranes.

1.4.2 Potentiometric biosensors

- Potentiometric biosensors make use of ion-selective electrodes in order to transduce the biological reaction into an electrical signal.
- It consists of an immobilised enzyme membrane surrounding the probe from a pH-meter (, where the catalysed reaction generates or absorbs hydrogen ions.
- The reaction occurring next to the thin sensing glass membrane causes a change in pH which may be read directly from the pH-meter's display.
- Here electrical potential is determined at very high impedance allowing effectively zero current flow and causing no interference with the reaction.

$Hg \leftrightarrow Hg^{+} + e-$
$HgCl_2 \leftrightarrow 2\,Hg^{+} + 2Cl^{-}$
$KCl \leftrightarrow K^{+} + Cl^{-}$

$Ag \leftrightarrow Ag^{+} + e-$
$AgCl \leftrightarrow Ag^{+} + Cl^{-}$
$HCl \leftrightarrow H^{+} + Cl^{-}$

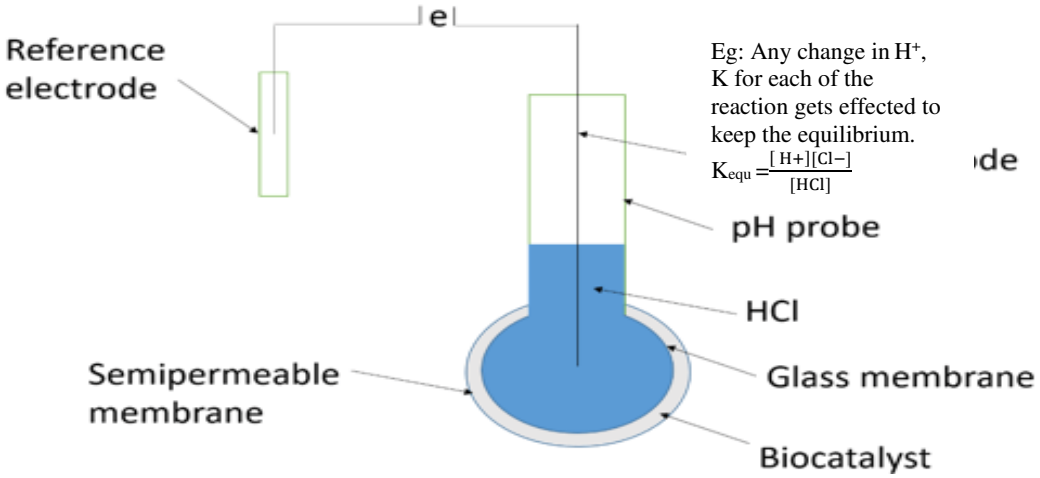


Fig 7. A simple potentiometric biosensor. A semi-permeable membrane surrounds the biocatalyst entrapped next to the active glass membrane of a pH probe.

The electrical potential is generated between the internal Ag/AgCl electrode bathed in dilute HCl and an external reference electrode. Semipermeable membrane surrounds the enzyme allows the analyte to move in. Potentials at reference electrode are unaffected by changes in H^+. Reaction generates or absorbs H^+ at glass electrodes. So, equilibrium of the reaction alters to keep the K constant for each of the reaction. Reaction. Changes in H^+ glass electrodes ultimately changes the electron concentration of the reaction. Measure of the current between two electrodes will be measure of H^+ of the solution.

1.4.3 Optical biosensors

- An optical biosensor is a compact analytical device containing a biorecognition sensing element integrated with an optical transducer system (Figure 8).
- The basic objective of an optical biosensor is to produce a signal which is proportionate to the concentration of a measured substance (analyte).
- The optical biosensor can use various biological materials, including enzymes, antibodies, antigens, receptors, nucleic acids, whole cells and tissues as biorecognition elements.
- Surface plasmon resonance (SPR), evanescent wave fluorescence and optical waveguide interferometry utilize the evanescent field in close proximity to the biosensor surface to detect the interaction of the biorecognition element with the analyte.
- There are a huge number of variations in the construction of optical biosensors.

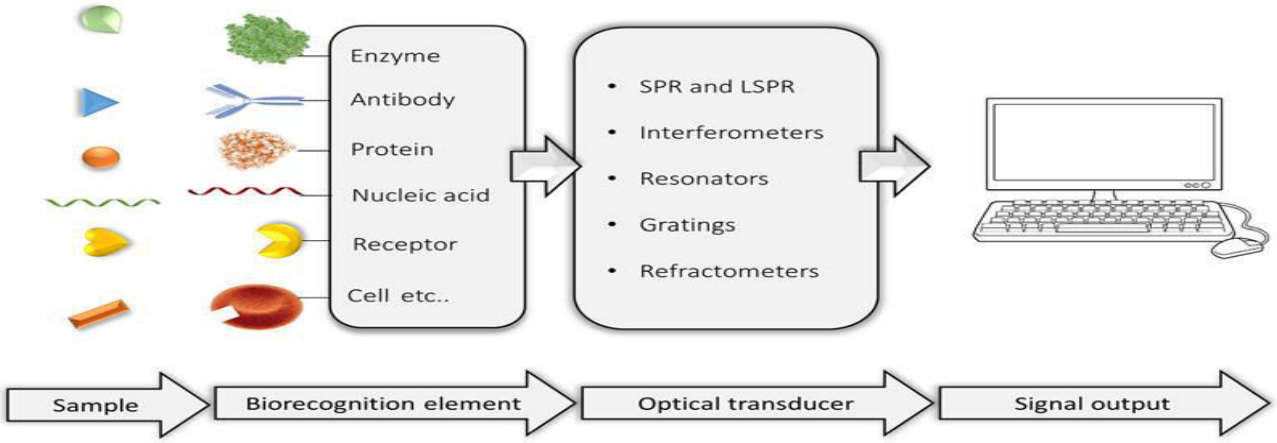


Figure 8 Generalised representation of optical biosensors.

Surface plasmon resonance biosensors (SPR)

- The SPR phenomenon occurs on the surface of metal (or other conducting materials) at the interface of two media (usually glass and liquid) when it is illuminated by polarized light at a specific angle.
- This generates surface plasmons and consequently a reduction of the intensity of reflected light at a specific angle known as the resonance angle.

- This effect is proportionate to the mass on the surface.
- A sensorgram can be obtained by measuring the shift of reflectivity, angle or wavelengths against time.
- In all configurations, the SPR phenomenon enables direct, label-free and real-time changes of refractive index at the sensor surface, which is proportionate to the biomolecule concentration.
- To measure a ligand–analyte interaction, one interacting molecule must be immobilized on the sensor surface.
- A practical SPR instrument combines an optical detector part, usually measuring intensity shift, a sensor chip with a gold surface and a layer enabling ligand immobilization, which is integrated with a fluidics system enabling a flow-through operation.
- The operating principle of a typical SPR instrument is presented in Figure 9

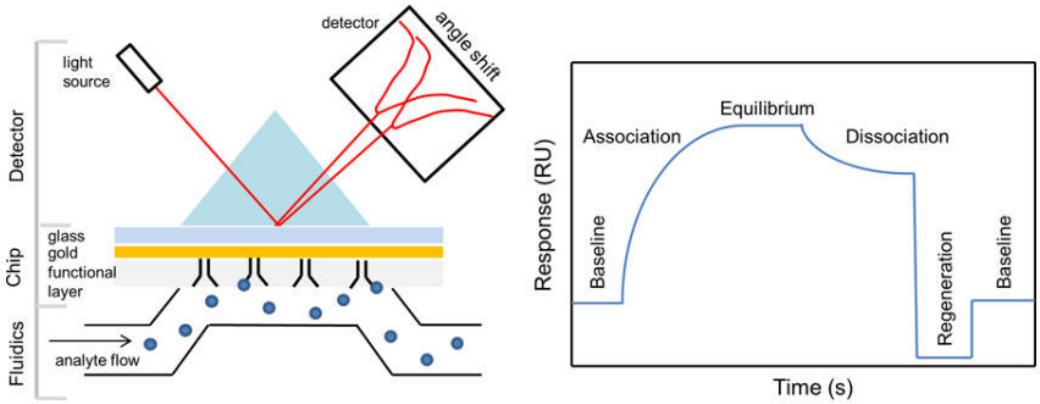


Figure 9: The operating principle of a typical SPR instrument

Surface plasmon resonance imaging (SPRi)

- SPR imaging (SPRi) takes the SPR analysis a step further by merging the sensitivity of SPR and spatial imaging in a microarray format allowing the simultaneous study of multiple different interactions.
- SPRi allows simultaneously studying multiple different interactions on an array of precisely patterned molecules (Figure 10).
- High throughput, sensitivity and obtaining the spatially resolved images of biointeractions open up a great future for SPRi to be applied in clinical chemistry and medicine for the screening of biomarkers and therapeutic targets.
- For example, a successful application of this method was the kinetic study of the binding between an immunosuppressive drug (FK506) and its target protein (FK506-binding protein 12 (FKBP12)) in a high-throughput SPRi format with a detection limit of 0.5 nM.

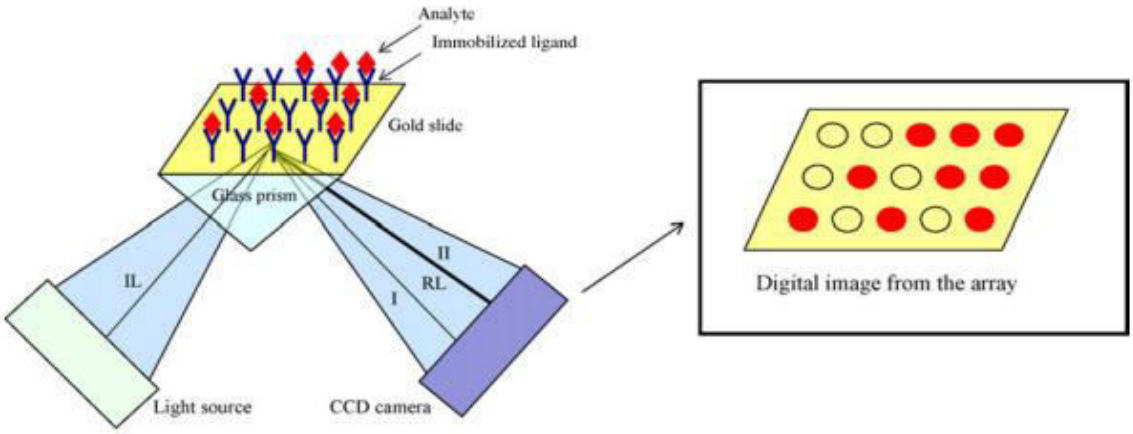


Figure 10: The operating principle of a typical SPRi

Localized surface plasmon resonance

- Localized SPR (LSPR) is based on metallic nanostructures (MNPs) (Au, Ag, etc.) having unique optical properties which are not seen in larger metal structures.
- A particularly striking example of such phenomenon is the red colour of aqueous dispersions of colloidal gold particles, which is a manifestation of LSPR.
- The optical phenomenon of LSPR occurs when incident light interacts with MNPs, the electromagnetic field of the light induces collective electron charge oscillations confined in MNPs and the subsequent absorbance of light within the ultraviolet–visible (UV-VIS) band (Figure 4).
- Thus, the major difference between SPR and LSPR is that induced p plasmons oscillate locally on thenanostructure rather than along the metal/dielectric interface (Figure 11).

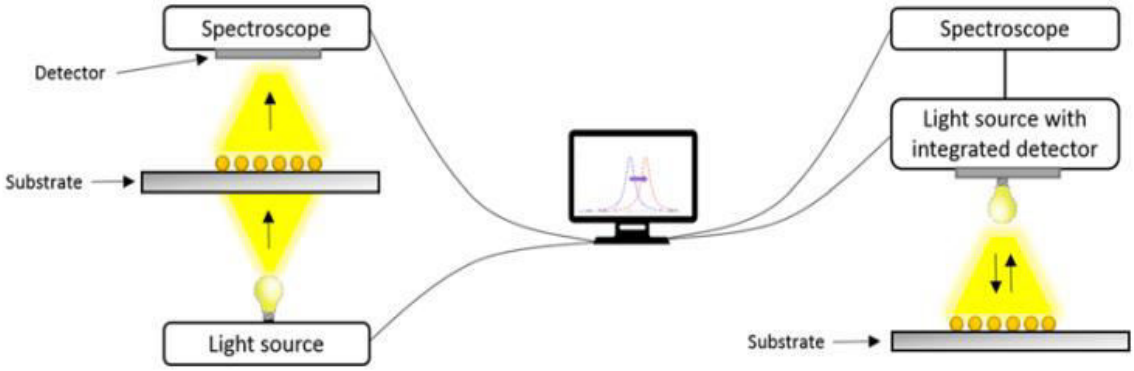


Fig 11 Localized surface plasmon resonance

Evanescent wave fluorescence biosensors

- In these biosensors, the biological recognition and the consequent binding event occur within the confines of an evanescent wave.
- The evanescent wave arises from the manner in which light behaves when confined in an optical waveguide or fibres.

- Guided light is totally internally reflected when it meets the interface of the waveguide/fibre and a surrounding medium with a lower index of refraction, as a result an electromagnetic field called an evanescent wave extends out from the interface into the lower index medium.
- The evanescent wave decays exponentially with distance from the surface, generally over the distance of 100 nm to approximately a wavelength.
- Since the evanescent wave is such a near-surface phenomena, detection employing evanescent wave excitation to generate the fluorescent signal is surface-sensitive, meaning that only fluorescent molecules near the surface are excited (Figure 12).

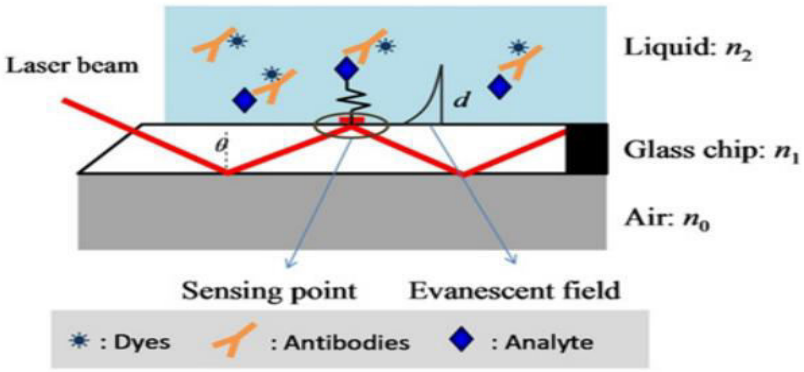


Fig 12 Evanescent wave fluorescence biosensors

- **Bioluminescent optical fibre biosensors:** This technique uses recombinant bioluminescent cells and the bioluminescent signal is transferred from the analyte by an optical fibre.
- **Optical waveguide interferometric biosensors**: An integrated planar optical waveguide interferometric biosensor is a combination of evanescent field sensing and optical phase difference measurement methods. By probing the near-surface region of a grating sensor area with the evanescent field, any change of the refractive index of the probed volume induces a phase shift of the guided mode compared with a reference field, typically of a mode propagating through the reference arm of the same waveguide structure. The interfering fields of these modes produce an interference signal detected at the sensor's output, whose alteration is proportional to the refractive index change and the signal is related to the concentration of the analyte.
- **Ellipsometric biosensors:** An ellipsometric biosensor measures changes in the polarization of light when it is reflected from a surface. This platform was applied in detecting the binding of influenza A virus strains with a panel of glycans of diverse structures.
- **Reflectometric interference spectroscopy biosensors:** Reflectometric interference spectroscopy (RIfS) is a label-free and time-resolved method where the simple optical setup is based on white light interference at thin layers. Changes in the phase and amplitude of polarized light provides information about the thickness and refractive index of the adsorbed protein layer.

- **Surface-enhanced Raman scattering biosensors:** Surface-enhanced Raman scattering (SERS) is a biosensing technique which enhances the intensity of the vibration spectra of a molecule by several orders of magnitude when it is in close proximity to nano-roughened metallic surfaces or nanoparticles made of gold or silver

1.4.4 Acoustic biosensors

- Bulk acoustic wave (BAW) biosensors employ either longitudinal or shear waves, although the latter is often preferred to reduce acoustic radiation in the medium of interest.
- They are the oldest and the simplest acoustic wave devices.
- BAW devices consist of a parallel electrode placed on both sides of the thin piece of crystal. BAW sensor can technically employ any piezoelectric element, and typically quartz is used, as it is an inexpensive material readily available in nature and easily synthesizable in abundant quantities.
- In addition, thin disks of quartz are more stable at high temperatures than other piezoelectric elements.
- When an alternating electric field is applied, it results in a potential difference between the two electrodes and the shear deformation of the crystal.
- As a result, there is mechanical oscillation of a standing wave across the bulk of the quartz.
- The frequency of the vibrations is dependent on quartz properties such as density, size, and phase in contact with the crystal surface.
- Currently, thickness shear mode (TSM) resonator and shear horizontal acoustic plate mode (SH-APM) sensors remain the most widespread BAW sensors (Fig 13).
- The other two common BAW sensors that lie beyond the scope of this entry are the thin rod acoustic wave sensors and the flexural plate wave devices.

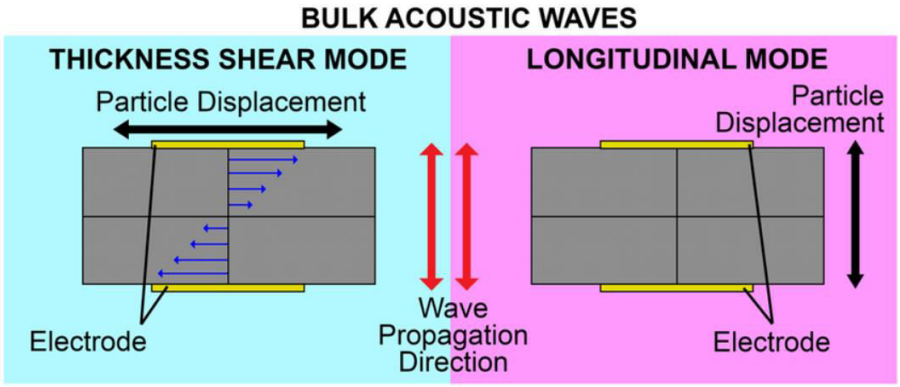


Fig 13 Modes of BAW. Thickness shear mode (TSM) and longitudinal mode

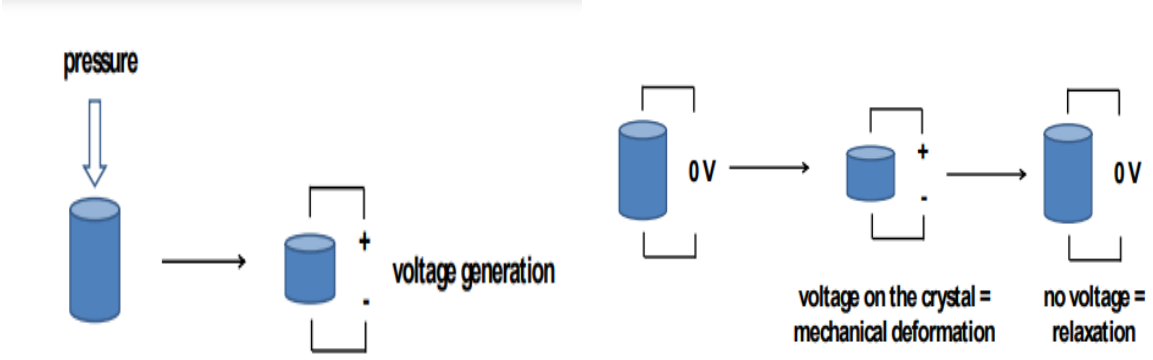
- **Thickness Shear Mode (TSM) Resonator:** TSM resonator, also known as quartz crystal microbalance (QCM), is the simplest and most widespread acoustic wave device today. TSM typically composes of a quartz plate sandwiched by electrodes on opposite faces. Electric field

crosses through this plate when voltage is applied to the electrodes, resulting in a shear mechanical strain or displacement in the quartz. By oscillating the voltage frequency, a mechanical resonance can be generated, where the maximum displacement of crystal occurs at the surfaces.

- **Shear Horizontal Acoustic Plate Mode (SH-APM) Sensor**:
  SH-APM sensors use a thin piezoelectric substrate, or a plate, to guide the acoustic wave and to confine its energy within the plate's top and bottom surfaces. Most of the production and analysis principles employed in SH-APM sensors are used in a TSM resonator. Their most striking difference is that SH-APM sensors employ inter digital transducers (IDT) rather than electrode plates. IDTs are deposited on opposite ends of a surface, where one IDT generates displacement waves through application of an oscillating voltage and the other receives it. The surface without IDT is immersed in the targeted liquid and acts as the sensor, so the device will not suffer from corrosion problems as electrode plates do in biological solutions.

1.4.5 Piezoelectric biosensors

- Anisotropic crystals i.e. crystals without centre of symmetry can generate electric dipole when mechanically squeezed.
- The electric dipole is also called piezoelectricity.
- The described effect can work in oppose way when an anisotropic crystal become deformed due to voltage imposed on it.
- The mechanical deformation is, however, a simple situation and oscillation is rather chosen in the common applications like here described analytical devices.
- In the case of oscillation, an alternating voltage is imposed on the crystal and mechanical oscillation then occurs



a b

Figure 14. Piezoelectric effect when voltage is generated because of mechanical deformation (a), when mechanical deformation is initiated by an applied voltage (b)

- The oscillations can have many appearances depending upon material and other conditions like electrical contacts, shape of the crystals etc.
- The oscillations occur in adiabatic waves which are typically spread over the mass like the acoustic one.
- In the oscillating crystals, the both surface acoustic waves spreading on the material and bulk acoustic waves occurring in deep matter can take place.
- In standard analytical applications, frequencies of oscillations are measured and interaction with either crystal alone or electrode leading electricity impulse on the crystal surface can serve for the determination of analyte.
- Piezoelectric biosensors are a group of analytical devices working on a principle of affinity interaction recording.
- A piezoelectric platform or piezoelectric crystal is a sensor part working on the principle of oscillations change due to a mass bound on the piezoelectric crystal surface.
- Biosensors having their surface modified with an antibody or antigen, with a molecularly imprinted polymer, with genetic information like single stranded DNA, and biosensors with bound receptors of organic of biochemical origin, are presented and discussed.
- **Piezoelectric Immunosensors:** The piezoelectric immunosensors are biosensors which contains an antibody as a biorecognition element and specificity of the antibody significantly influences specificity of the whole immunosensor.

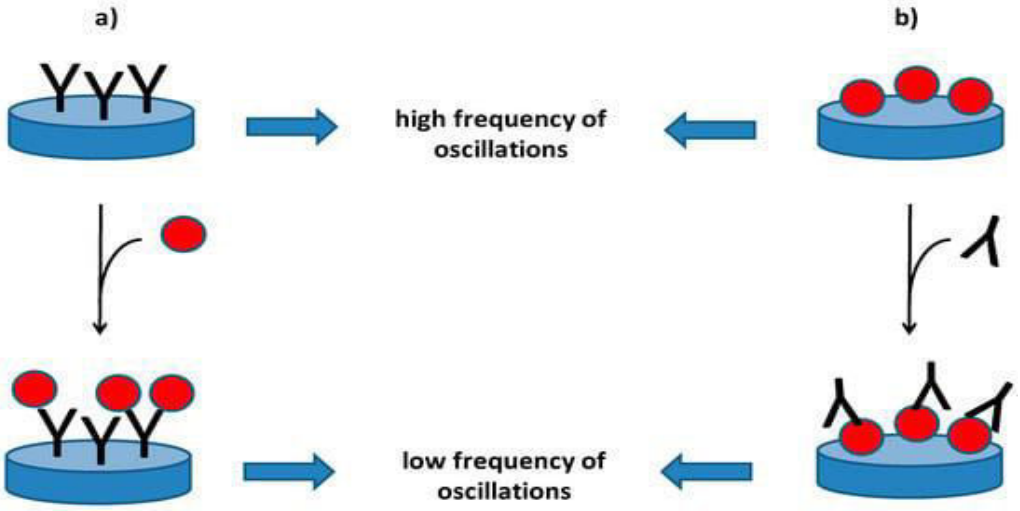


Figure 15. Piezoelectric immunosensors for the determination of an antigen (a) or an antibody(b)

- **Molecularly Imprinted Polymers on Piezoelectric Platform:** Molecularly Imprinted Polymers are specific artificial materials that can substitute antibodies or antigens as a biorecognition part in a biosensor.

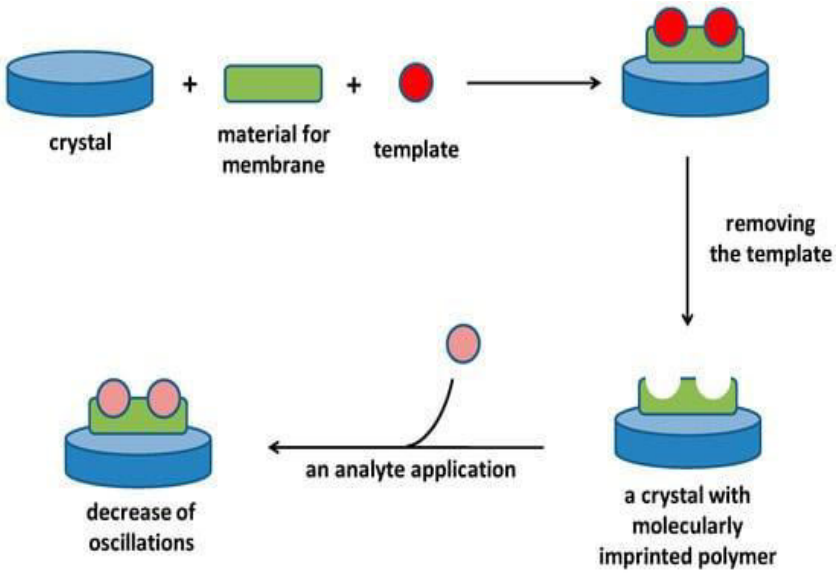


Figure16. Covering of crystal with a Molecularly Imprinted Polymer and following assay of an analyte chemically identical or close to the template.

- **Genetic Information Using Piezoelectric Biosensors:** Genetic information can be employed as a biorecognition part of various biosensors. Single-strand short strains of DNA or RNA can by written down as typical examples of genetic information forms that are suitable for biosensors construction.

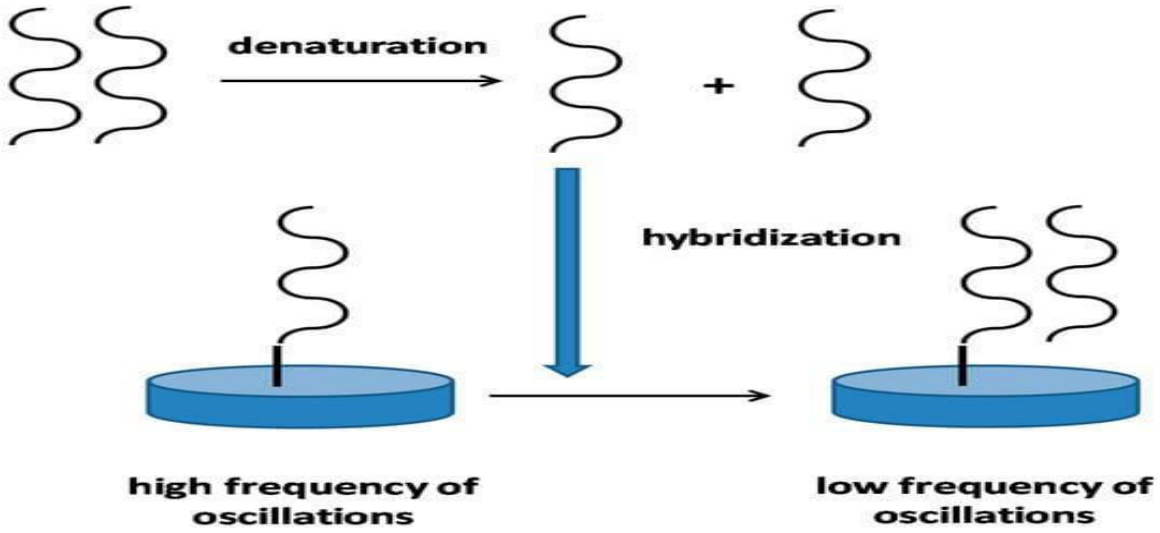


Figure 17. DNA piezoelectric biosensor.

1.4.6 Calorimetric biosensors

- Many enzyme catalysed reactions are exothermic, generating heat which may be used as a basis for measuring the rate of reaction and, hence, the analyte concentration.

- This represents the most generally applicable type of biosensor.
- The temperature changes are usually determined by means of thermistors at the entrance and exit of small packed bed columns containing immobilised enzymes within a constant temperature environment (Figure 18).
- Under such closely controlled conditions, up to 80% of the heat generated in the reaction may be registered as a temperature change in the sample stream.
- This may be simply calculated from the enthalpy change and the amount reacted.
- Calorimeter sensors are widely used in different areas such as biochemical, clinical, and pharmaceutical industries, and many more.
- In the calorimetry sensor, the energy released during a biochemical reaction is calculated as the measure of the interaction of the tested molecules.
- Two processes are used with the calorimetry sensor: (1) Adiabatic calorimetry, where there is no heat exchange between the external environment and the reaction vessel, and (2) Heat conduction calorimetry, which involves the heat transfer from a vessel to the surrounding heat sink.

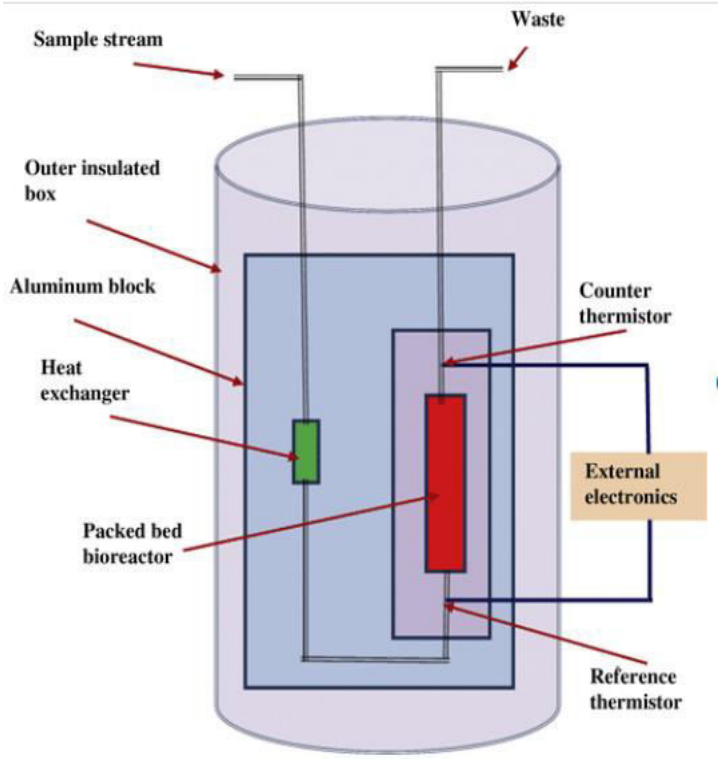


Figure 18. Calorimetric biosensors. Schematic diagram of a calorimetric biosensor. The sample stream passes through the outer insulated box to the heat exchanger within an aluminium block. From there, it flows past the reference thermistor and into the packed bed bioreactor, containing the biocatalyst, where the reaction occurs. The change in temperature is determined by the thermistor and the solution passed to waste. External electronics (l) determines the difference in the resistance, and hence temperature, between the thermistors.

1.5 Scope of biosensors and its limitations.

- There is a huge application of biosensor in the medical industry for testing purpose.
- The biosensor is used for pathogens detection purpose.
- Biosensors are also used for water treatment purpose.
- Biosensors are used for environmental monitoring purposes such as gas detection or tracing.
- They used for toxic metabolites detection.
- The biosensor is used to identify the contaminants in water such as heavy metal ions. This process is generally used when river water is treated for drinking.
- Biosensors are used in the security system and biodefense technology.
- Blood Glucose biosensor is used for glucose monitoring inside the human body.
- The biosensor also used for testing of cholesterol.
- Biosensors are used for the measurement of vitamins, biotin, folic acid, etc.
- Biosensors are used in Agriculture, Biotech industries to continuously monitor types of chemical, their properties in a specific system.

Biosensors	Transduction	Advantages	disadvantages	Applications
Amperometric and Potentiometric	Oxygen electrode	- simple - very specific (more selective)	- low sensitivity	glucose, sulfites, glutamate, ethanol, sucrose, lactate, cholestérol…
Amperometric	Hydrogen peroxide electrode	- simple - faster, more sensitive, more precise and more accurate than potentiometric biosensors	- little specific (less selective)	sulfites, glucose, glutamate, amino acids
Potentiometric	Hydrogen peroxide electrode	- simple - sensitive - very short response times	- not very specific (less selective)	sulfites, glucose, glutamate, amino acids
optical	Optical systems	- remote use - low costs - miniaturizable - no electrical interference	- need for high energy sources - narrow concentration range - Interference of incident light.	nitrate, glucose, glycerol, ethanol, galactose…
calorimetric	Calorimetric	no optical interference like color and turbidity	-expensive, - bulky - need for large amounts of enzyme	glucose, lactate, ascorbic acid, cholesterol, galactose, ethanol, lipids.

Table 1: Scope of biosensors and its limitations

Beneficial Features: A successful biosensor must possess the following features:

- The biocatalyst must be highly specific for the purpose of the analyses be stable under normal storage conditions except in the case of calorimetric enzyme strips and show good stability over a large number of assays (i.e. much greater than 100).
- The reaction should be as independent of physical parameters as like stirring, pH and temperature.
- The response should be accurate, precise, reproducible and linear over the useful analytical range without dilution or concentration. It should also be free from electrical noise.
- If the biosensor is to be used for invasive monitoring in clinical situations the probe must be tiny and biocompatible having no toxic or antigenic effects. If it is to be used in fermenters it should be sterilisable. This is preferably performed by autoclaving but no biosensor enzymes can presently withstand such drastic wet-heat treatment. In either case the biosensor should not be prone to fouling or proteolysis.
- The complete biosensor should be cheap, small, portable and capable of being used by semi-skilled operators.
- The biosensors have been considered to be superior and more sensitive, in comparison to physical instruments due to the following reasons:
  - In a biosensor the immobilized biological material is present in intimate contact of a suitable transducer so that the biochemical signal is quickly converted into an electrical signal.
  - The immobilization of biomolecules permits reuse of these molecules (which are expensive) and allows simplification of the entire apparatus.
  - The biological sensing element is present in a small area and is very sensitive, thus facilitating analysis of substances in small quantities.
  - Biosensors may be developed according to specific needs and can be highly specific or show broad spectrum**.**

Disadvantages:

- Heat sterilization is not possible as this would denature the biological part of the biosensor.
- The membrane that separates the reactor media from the immobilized cells of the sensor can become fouled by deposits.
- The cells in the biosensor can become intoxicated by other molecules that are capable of diffusing through the membrane.
- Changes in the reactor broth (i.e., pH) can put chemical and mechanical stress on the biosensor that might eventually impair it.

Module 2

BASIC DESIGN AND TRANSDUCER

2.1 Design Considerations: calibration, dynamic Range, signal to noise, sensitivity, selectivity, Interference recognition.

2.2 Transduction membrane protein sensors: ion channels,

2.3 Types of Transducer, Optical; Fiber Optic, ECL, Surface Plasmon Resonance, Electro chemical; FET, Impedance, Piezoelectric; Cantileaver,

2.1 Design Considerations: Calibration, Dynamic Range, Signal to Noise, Sensitivity, Selectivity, Interference Recognition.

- A successful biosensor is composed of two main components, mainly a biological receptor or sensor element and a transducer.
- The first step in developing a biosensing device involves investigating the target analyte and understanding how this analyte interacts with certain biological molecules.
- Once this has been established, the following tasks are critical: Selection of a biological receptor: the specificity and selectivity of a biosensor to the analyte of interest is dependent upon the biological receptor used.
- A suitable receptor with high affinity for the analyte is thus recommended.
- Having knowledge of the advantages and disadvantages of various biological receptors in different biosensor applications is very important in selecting a suitable receptor.
- **Selection of a suitable immobilization method**: for any biological molecule to operate reliably as a biological receptor, it requires attachment onto the surface of a transducer, the process is known as immobilization.
- Various methods have been used for this task and include adsorption, entrapment, covalent attachment, micro encapsulation and cross linking.
- **Selection of a transducer element**: the transducer element greatly influences the sensitivity of the biosensor device.
- Employing the right transducer will result in a device with increased sensitivity while the sensitivity is more likely to be compromised by the use of an ineffective transducer

2.1.1 Calibration

- Sensor Calibration in simple terms can be defined as the comparison between the desired output and the measured output.
- On-site monitoring requires enhanced sensitivity, selectivity, rapidity, and ease of operation of the analytical equipment, which should provide reliable continuous information in real-time and demonstrate sufficient stability of action.
- We use different systems and types of equipment for measuring various physical quantities.
- The accuracy of the measurement depends upon various factors.
- The equipment used for measurements can lose their precision when used at higher temperatures, high moisture or humidity conditions, subjected to degradation, subjected to external shocks, etc.This can be observed as the error in the measurement.
- To tackle this error and make necessary changes to the equipment calibration methods are used. Today sensors are being used for making various measurements.
- There are sensors to measure temperature, colour, humidity, etc…Sensor Calibration plays a crucial role in removing the errors in sensor measurements.
- Sensors are electronic devices. They are sensitive to the changes in their working environment. Undesirable and sudden changes in the working environments of the sensors give undesired output values. Thus, the expected output differs from the measured output. This comparison between the Expected output and measured output is called Sensor Calibration.
- Sensor calibration plays a crucial role in increasing the performance of the sensor. It is used to measure the Structural errors caused by sensors. The difference between the expected value and the measured value of the sensor is known as the Structural Error.
- Sensor calibration helps in improving the performance and accuracy of the sensors.
- There are two well-known processes in which sensor calibration is done by industries.
- In the first method companies **add an In-house calibration process** to their manufacturing unit to perform individual calibration of the sensors. Here the company also adds necessary hardware to their design for sensor output correction. By this process, the sensor calibration can be changed to match the application-specific requirements. But this process increases the time to market.
- The alternative of this **In-house calibration process**, several manufacturing companies provides sensor packages with a high-quality automotive-grade MEMS sensor along with complete system-level calibration. In this process, the companies include an on-board digital circuitry and software to help designers to improve the functionality and performance of the sensors.
- To reduce the product design time and component count, digital circuitry such as voltage regulation and Analog signal filtering techniques are included.

- To improve the overall performance and functionality, the on-board processor is provided with sophisticated sensor fusion algorithms.
- Some of the sophisticated on-board signal processing algorithms also help in reducing the manufacturing time enabling the faster time to market.

Standard Reference Method

- Here the sensor output is compared with a standard physical reference to know the error in some sensors. Examples of sensor calibration are rulers and meter sticks, For temperature sensors- Boiling water at 100C, Triple point of water, For Accelerometers- "gravity is constant 1G on the surface of the earth".

Calibration Methods:

- There are three standard calibration methods used for sensors. They are-**One point calibration, Two-point calibration, and Multi-Point Curve Fitting**.
- Before knowing these methods we have to know the concept of Characteristic curve.
- Every sensor has a characteristic curve that shows the response of the senor to the given input value. In the calibration process, this characteristic curve of the sensor is compared with its ideal linear response.
- Some of the terms used with the characteristic curve are-
- **Offset** – This value tells us whether the sensor output is higher or lower than the ideal linear response.
- **Sensitivity or Slope** – This gives the rate of change of sensor output. A difference in slope shows that the sensor output changes at a different rate than the ideal response.
- **Linearity** – Not all sensors have a linear characteristic curve over the given measurement range.
- One point calibration is used to correct the sensor offset errors when accurate measurement of only a single level is required and the sensor is linear.
- Temperature sensors are usually one point calibrated.

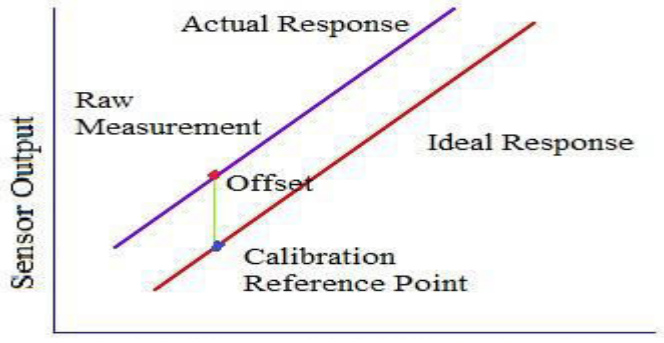


Fig 19: One-Point-Calibration

- Two-point calibration is used to correct both slope and off-set errors. This calibration is used in the cases when the sensor we know that the sensor output is reasonably linear over a measurement range. Here two reference values are needed- reference high, reference Low.

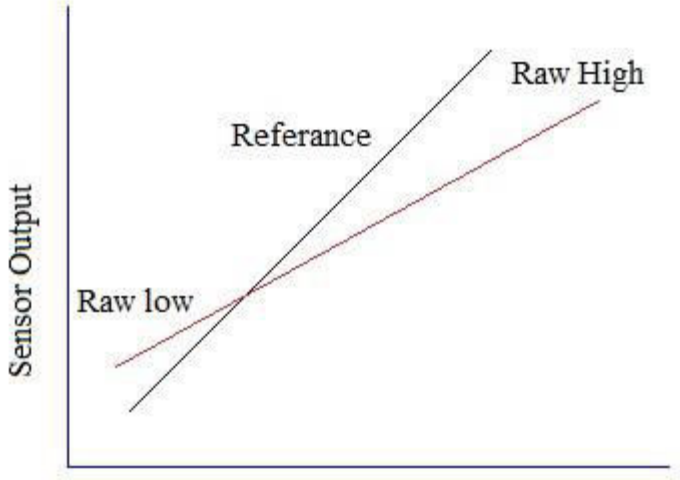


Fig 20 Two-Point-Calibration

- Multi-point Curve fitting is used for sensors that are not linear over the measurement range and require some curve-fitting to get the accurate measurements.
- Multi-point curve fitting is usually done for thermocouples when used in extremely hot or extremely cold conditions.
- For all the above calibration process, the characteristic curves of the sensors are drawn and compared with the linear response and error is known.
- The calibration process helps us to determine the following results-No error noted on the DUT,
- An error is noted and no adjustment is made.
- An adjustment is made to remove the error and the error is corrected to the desired level.
- For sensor calibration sensor models are used. Sensor calibration is applied in Control systems to monitor and adjust the control processes. Automatic systems also apply the sensor calibration to get error-free results.
- Use of Sensor Calibration: The calibration process is used to increase the performance and functionality of the system. It helps in reducing errors in the system. A calibrated sensor provides accurate results and can be used as a reference reading for comparison.

2.1.2 Dynamic range

- Dynamic range is one of the design parameter, when digital sensors are component of sensor device.

- Here, dynamic range of measurement will be also related to the number of binary digits (bits) used in a digital numeric representation in which the measured value is linearly related to the digital number.
- Often this dynamic range of measurement is limited at one end of the range by saturation of a sensing signal sensor or by physical limits that exist on the motion or other response capability of a mechanical indicator.
- The other end of the dynamic range of measurement is often limited by one or more sources of random noise or uncertainty in signal levels that may be described as defining the sensitivity of the sensor or metrology device.
- The range of concentrations over which the biosensor exhibits a change in output is the operational range of the biosensor.
- Operational range is determined by measuring the concentrations over which the biosensor shows a graded, concentration-dependent change in response (Figure 21).

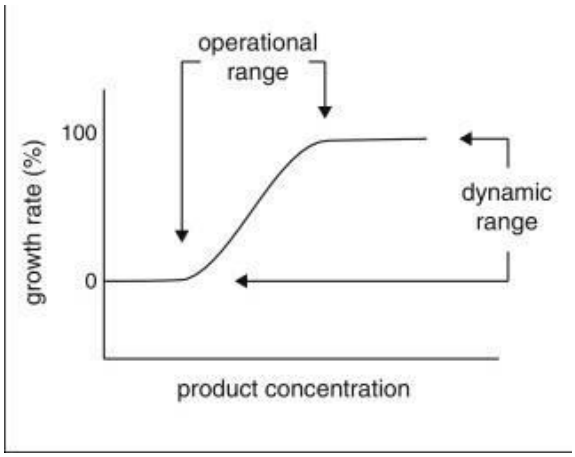


Fig 21 Biosensor transfer function

- Defining engineering parameters for biosensors is a prerequisite for biosensor-based measurements.
- The relationship between biosensor output and product concentration is the biosensor transfer function.
- The range of concentrations over which the biosensor functions is the operational range. The intensity of the biosensor response is the dynamic range.
- Sensor dynamic range quantifies the ability of a sensor to acquire both high and low signals.
- It is defined as the ratio of the largest no saturating input signal to the smallest detectable input signal.
- The false positive rate of a biosensor system determines the maximum number of designs that can be evaluated in a given experiment.

- The signal-to-noise of a biosensor, also referred to as the dynamic range of the system, can be quantified as the ratio of the highest measured output of the biosensor to the lowest measured output of the biosensor.
- Dynamic range is the ratio between the largest and smallest values that a certain quantity can assume.
- Various strategies are used to tune, extend, and narrow the dynamic range of Biosensors that use either optical or electrochemical readouts.
- These strategies enable one to tune the affinity of biosensors that detect nucleic acids, small molecules, heavy metal ions, pH, and temperature.
- In addition, environmental changes and material sizes are also used to adjust the dynamic range of Biosensors.
- However, biosensors with extended dynamic range display reduced precision, while highly precise sensors display narrowed dynamic range.
- Biosensor design strategy should be to develop dual-signalling biosensor architecture that simultaneously provides both a highly sensitive "signal-on" readout over a small fixed dynamic range as well as a "signal-off" readout, enabling quantification over a large, extended dynamic range.

2.1.3 Signal-to-noise ratio

- In quite many applications of sensors, noise adds to the useful signal, while in others, noise is proportional to signal.
- Whatever the case, the larger the noise fluctuations, the more difficult it is to detect or measure the useful signal.
- Figure 22 shows a typical detector output with respect to time shows that the performance of an electro-optical sensor does not depend on its signal alone but on its signal to noise ratio.

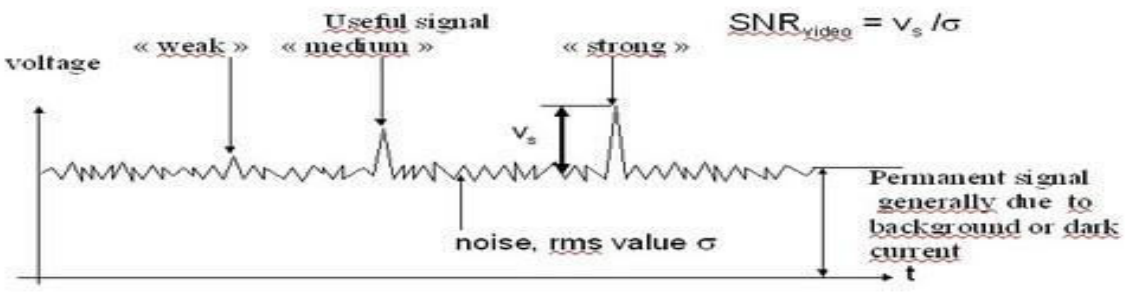


Figure 22: Signal to noise ratio

- Since the sensor output is noisy, it fluctuates above and below its average value by an instantaneous amount, for example ib (t) if one is concerned with the output current. The corresponding current or voltage variances, σ2i or σ2v, inside the electronic bandpass of the sensor generate the following electrical noise power Pn across the load resistor RL:
- If it is the instantaneous pertinent output from the detector, the corresponding electric power of the signal is :
- By definition, the power signal to noise ratio of the sensor at that corresponding instant is the ratio between the electrical pertinent signal power and that of the noise, both being evaluated inside the sensor bandpass:
- Spatial (geometric) and spectral filtering are aimed at minimizing shot noise, due for example to stray light, and at maximizing lens transmittance for the pertinent signal.
- Spatial filtering eliminate stray light from intense sources of light outside the field of view, by means of diaphragms, baffles, or protective screens.
- Spectral filtering separates useful from parasitic radiations.
- In Electronic filtering after detection, if the variation in time of the expected signal is known, and this signal is band limited and if the noise spectrum is white, signal processing techniques such as matched filtering are a good choice.

2.1.4 Sensitivity

- The sensitivity of the sensor is defined as the slope of the output characteristic curve (DY/DX in Figure 23) or, more generally, the minimum input of physical parameter that will create a detectable output change.
- In some sensors, the sensitivity is defined as the input parameter change required to produce a standardized output change.
- In others, it is defined as an output voltage change for a given change in input parameter. For example, a typical blood pressure transducer may have a sensitivity rating of 10 mV/V/mm Hg; that is, there will be a 10-mV output voltage for each volt of excitation potential and each mm Hg of applied pressure.
- The sensitivity is the derivative of the output with respect to the stimulus. For a transfer function, A, the sensitivity, b, for a particular input value B, so, is given by;
- $b = \left.\frac{dA}{dB}\right|_{s_o}$
- Therefore, in basic terms, the sensitivity is simply the smallest fractional change in a device that can be measured.

- Sensitivity is considered as the most important characteristic of a biosensor.
- The sensitivity of a biosensor is defined as the relationship between the change in analyte concentration and the intensity of the signal generated from the transducer.
- Ideally, a biosensor should generate a signal in response to small fluctuations in the concentration of the target analyte.
- Depending on the application, biosensors are required to detect analytes in the ng/ml or fg/ml concentration ranges.
- This is usually important for medical applications and environmental monitoring purposes.

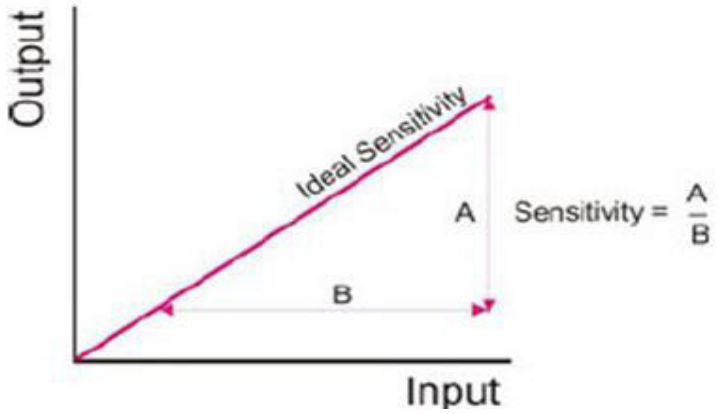


Figure 23: Illustration of sensitivity in biosensor

2.1.5 Selectivity

- This refers to the ability of the biosensor to selectively bind and respond only to the desired analyte, in the presence of other molecules or substances.
- When a signal or response is generated from interactions with an analyte that is different from the target analyte such is termed a false positive result.
- This is common in biosensors with poor selectivity, thus failing in clinical applications.
- Selectivity is a very important feature especially in medical applications where the test sample or sample matrix, usually blood or urine, contains numerous molecules that are quite similar to the target analyte and compete for binding to the biological receptor.
- The selectivity of a sensor is the ability to discriminate the target from the interference molecules and display a target-specific sensor response.
- It is a critical trait for chemical sensors that are used in real-time air pollution control, hazardous materials detection, food quality inspection and personal health monitoring.
- Attaining high target selectivity ensures that sensors will exhibit accurate information about the existence and concentration of a target gas, which is essential for reliable sensor response.

- To obtain target selectivity, it is critical to determine the optimum modification technique and receptor materials as well as to understand how each method works and how it could be designed for a specific target.

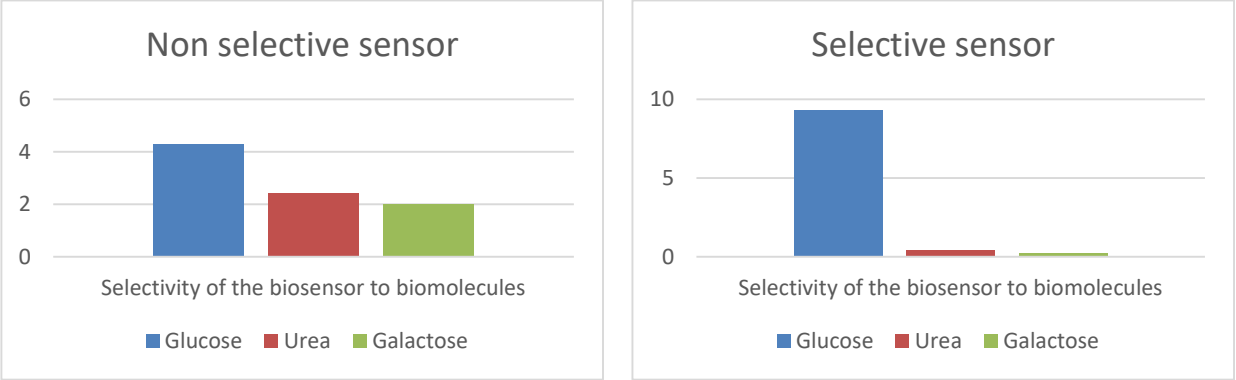


Figure 24: Selectivity test of sensor with respect to different biomolecules

2.1.6 Interference recognition

- Sensor systems are used every time a microcontroller needs to interact with the physical world.
- They are abundant in home automation, factory control systems, critical infrastructure, transport systems and many, many other things.
- In a sensor system, a sensor transforms a physical quantity into an analog signal which is sent to an ADC and a microcontroller for digitization and further processing.
- Once the measurement is in digital form, the microcontroller can execute tasks according to the measurement.
- Electromagnetic interference (EMI) can affect a measurement as it is transferred to the microcontroller.
- An attacker can manipulate the sensor output by intentionally inducing EMI in the wire between the sensor and the microcontroller.
- The nature of the analog channel between the sensor and the microcontroller means that the microcontroller cannot authenticate whether the measurement is from the sensor or the attacker.
- If the microcontroller includes incorrect measurements in its control decisions, it could have disastrous consequences
- The active sensors communicate by transmitting probe signals.
- The communication of probe signals may result in cross-interference which may vary in time.
- Cross-interference is detected, and can later be avoided, by determining a difference between signals received in a first part of a timeslot and signals received in a second part of the timeslot.

- In order to do so probe signals comprising two non-zero pulses are transmitted in respective parts of the timeslot.
- Applications are, for example, active presence sensors in lighting control applications in indoor as well as outdoor environments.
- It has been discovered that cross-interference across active sensors (such as sensors based on ultrasound, or radio frequency) is a problem in indoor as well as outdoor sensing applications.
- Cross-interference across active sensors generally depends on the dimensions of the monitored space and presence/absence of objects therein.
- For instance, when an object is moved (or added/removed) the cross-interference pattern across sensors tends to vary.
- This affects proper operation of the presence sensing systems.

2.2 Transduction membrane protein sensors: ion channels

- Biological membranes are one of the essential components of living organisms, forming physical boundaries in biological cells, such as the plasma membrane and the organelle membranes.
- The principal components of membranes are phospholipids and membrane proteins.
- Phospholipids are amphiphilic molecules consisting of a hydrophilic head group and hydrophobic tails.
- They form a bilayer-membrane configuration in aqueous environments, which is attributed to the hydrophobic interactions of their hydrocarbon chains.
- Lipid bilayer membranes function as hydrophobic barriers against soluble and ionic molecules and prevent the entry of such molecules into the cytoplasm and organelles.
- Membrane proteins are incorporated in the lipid bilayer and allow signal transduction and transport of ligand molecules across the membrane.
- Binding of an odorant molecule to the ionotropic receptor directly triggers the influx of cations into the cell through the membrane; this influx stimulates the neurons.
- Since these protein receptors act as ligand-sensing elements, numerous studies have attempted to use this sensing property of the highly specific membrane receptors for the development of biosensors.
- Moreover, sensory systems often include a mechanism of input amplification that enhances the output signal-to-noise ratio.
- Biosensors that embed sensory systems are based on two major platforms: the lipid bilayer-based platform and the cell-based platform (figure 24).

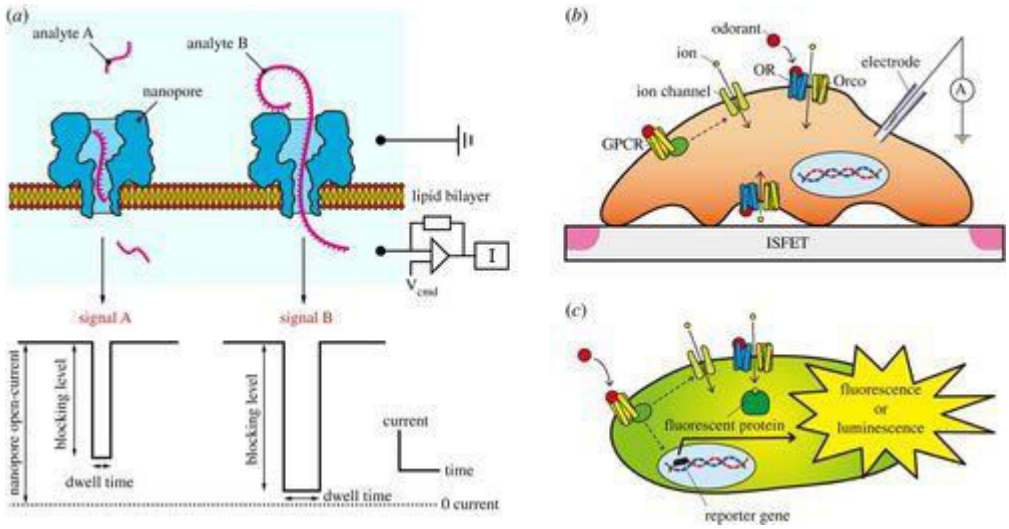


Figure25. Membrane protein-based biosensors

- Figure 1. Membrane protein-based biosensors on (a) a lipid-bilayer platform and (b,c) cell-based platforms. On the lipid bilayer platform, the nanopore protein is incorporated in the lipid bilayer. Single analyte molecules are detected based on the signatures of the current trace that translates the interaction between the analytes and the nanopore. On the cell-based platforms, such as those for odorant sensing, cell responses to odorants can be determined by (b) measuring the electrical alterations of the cell using electric signal measuring systems, including ion-sensitive field effect transistor (ISFET), (c) detection of fluorescence or luminescence changes initiated by olfactory stimuli. In this case, utilized cells express G protein-coupled receptor (GPCR) or olfactory receptor (OR) with OR co-receptor (Orco). (Online version in colour.)

Direct and indirect transduction

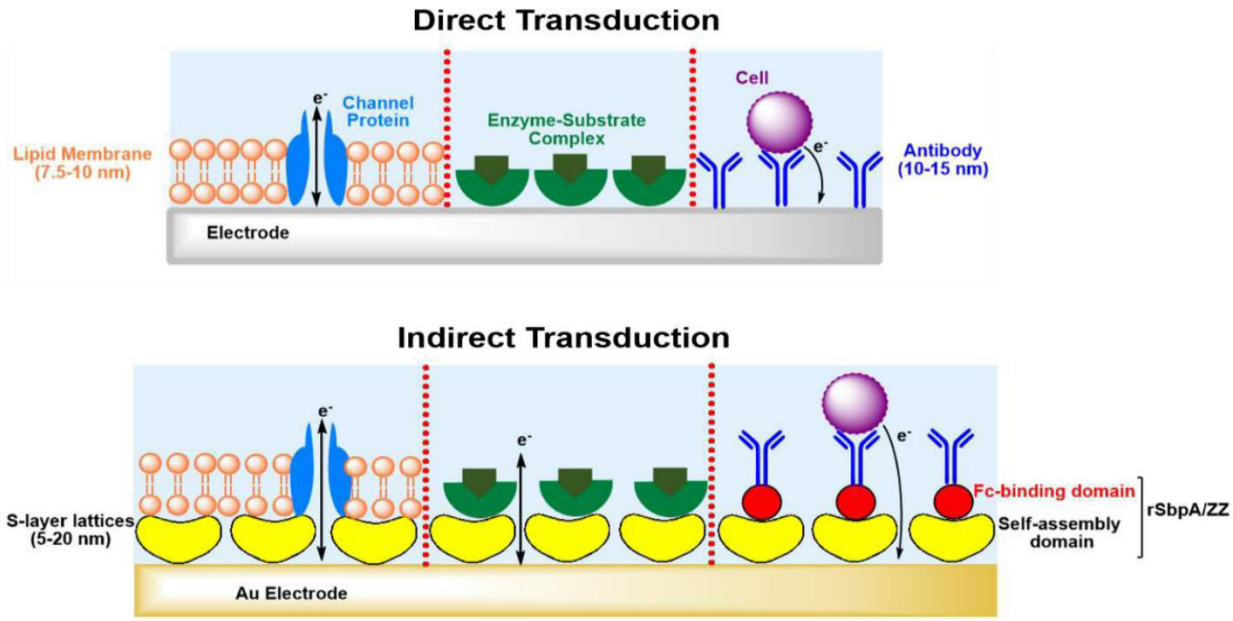


Fig: 26 Direct and indirect transduction

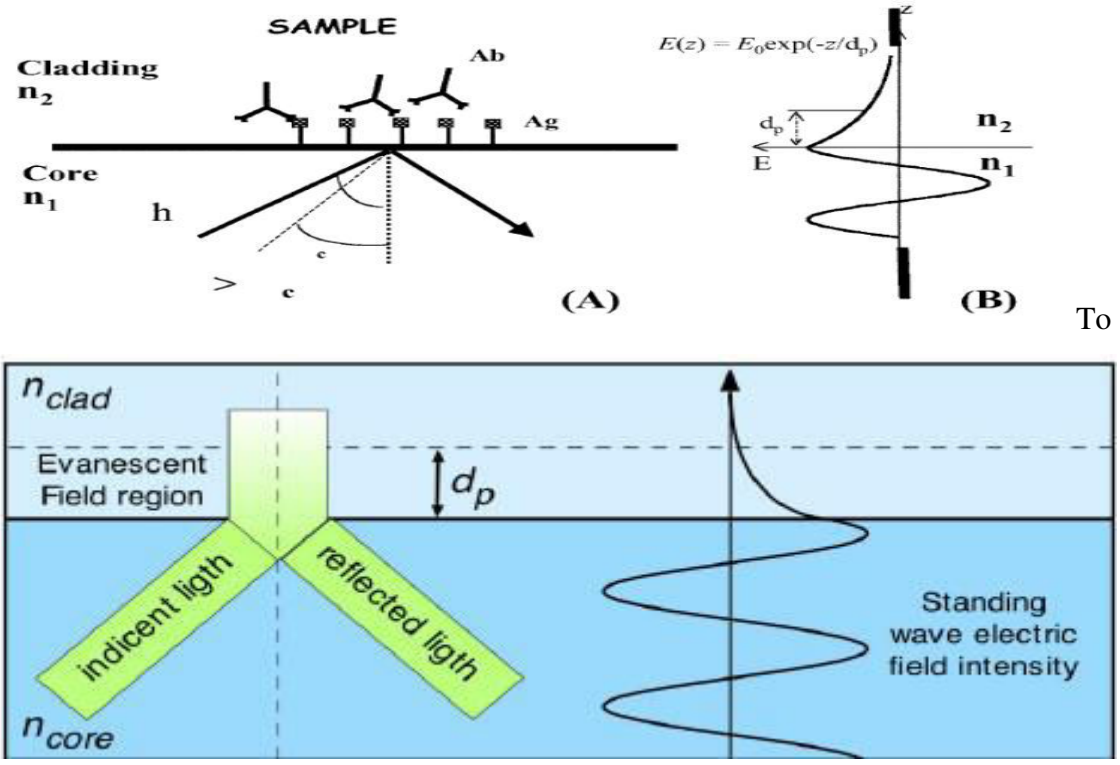
- In direct approach, the electron transfers are close to the surface, whereas in the indirect one, electron shuttles between the reaction site and the sensor surface.
- In indirect approach, The S-layer protein lattice constitutes an intermediate matrix. In the lipid-based biosensor (left), electrons transfer from the outer membrane to the inner membrane and vice versa via a channel protein.
- In the detection biosensor (middle and right), electrons transfer between the enzyme–substrate complex and cell/antibody and electrode surface, respectively. The S-layer lattice provides an immobilization matrix and ion reservoir.
- The pores of the S-layer lattice ensure no impact on the electron transfer. Fc: fragment crystallizable; rSbpA/ZZ: recombinant S-layer protein from *Lysinibacillus sphaericus* CCM 2177 with fused Fc-binding Z-domain (synthetic analog of immunoglobulin G (IgG-binding B—domain) of protein A of *Staphylococcus aureus*).

2.3 Types of Transducer, Optical; Fiber Optic, ECL, Surface Plasmon Resonance, Electro chemical; FET, Impedance, Piezoelectric; Cantilever,

2.3.1 Fibre optic Biosensors

- Optical fibers transmit light on the basis of the principle of total internal reflection (TIR).
- When this phenomenon occurs the light rays are guided through the core of the fiber with very little loss to the surroundings.
- The optical fiber is formed by a core with a refractive index n1and a cladding with a refractive index n2.
- For light propagation by TIR the refractive index of the core (n1) must be larger than that of the cladding (n2), i.e. n1>n2.
- When a ray of light strikes the boundary interface between these transparent media of different refractive index and the angle of incidence is larger than the critical angle, defined by the Snell's law ($\theta c = \sin -1[n2/n1]$), it will be totally internally reflected and propagated through the fiber.
- When the incident light is totally internally reflected, its intensity does not abruptly decay to zero at the inter-face.
- A small portion of light penetrates the reflecting medium by a fraction of wavelength, far enough for recognition of the different refractive index.
- This electromagnetic field, called the evanescent wave, has an intensity that decays exponentially with distance, starting at the interface and extending into the medium of lower refractive index.

- The penetration depth (dp), defined as the distance required for the electric field amplitude to fall to 1/e (0.37) of its value at the interface, increases with closer index matching and it is also a function of the wavelength of the light and the angle of incidence.
- The evanescent wave can interact with molecules within the penetration depth, thereby producing a net flow of energy across the reflecting surface in the surrounding medium (i.e. that with refractive index n2) to maintain the evanescent field.
- This transfer of energy will lead to attenuation in reflectance which can be used to develop absorption sensors based on evanescent waves (attenuated total reflection (ATR) sensors).
- When the evanescent lights electively excites a fluorophore, the fluorescence emitted can be directed back into the fiber and guided to the detector.



To

Fig: 27 Total Internal refraction [a] and electrical field amplified[b].

2.3.2 Electro chemiluminescence Biosensor (ECL)

- Electrochemiluminescence (ECL) is a chemiluminescence phenomenon resulting from the electrochemical excitation of a luminescence system (luminophore) that emits light when it returns to its fundamental state.
- The mechanisms associated with these phenomena are classified into two main types, annihilation pathway and Co-reactant pathway (Fig 26).

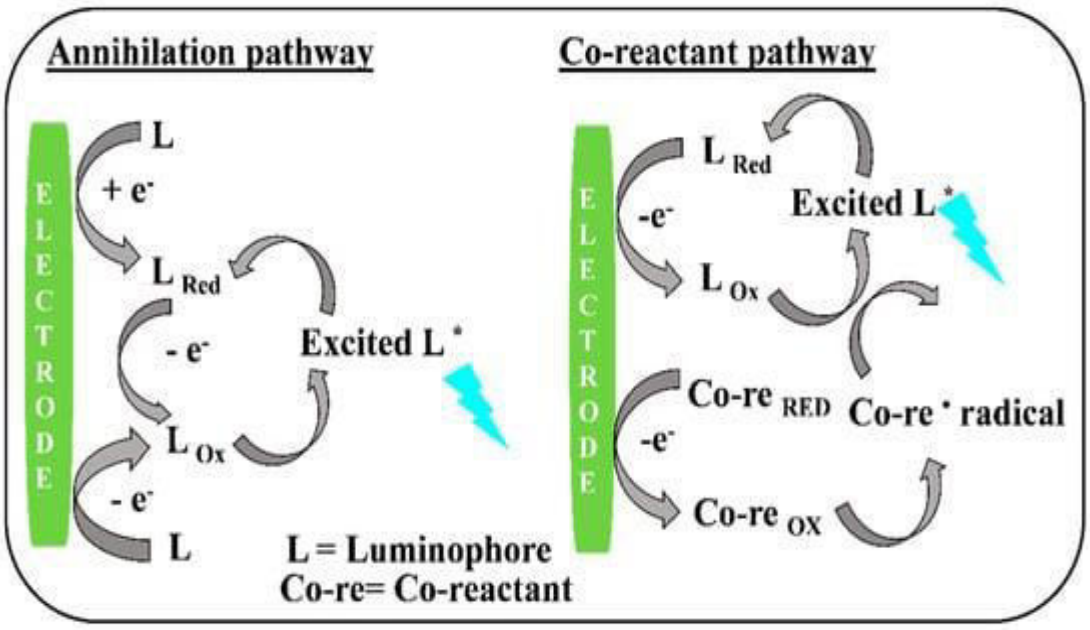


Fig 28: Mechanisms associated with Electrochemiluminescence

The annihilation pathway:

- A reduced specie and an oxidized specie (charged radical ions) are simultaneously generated at the electrode surface by applying alternating pulse potentials.
- These two species react between them generating an excited form, which in the relaxation process to the ground state emits a photon.

Co-reactant pathway:

- A co-reactant is a chemical specie that is reduced or oxidized at the electrode surface, generating a very reactive intermediates that react with the reduced or oxidized luminophore (specie capable of emit light) present in the solution to produce the excited state.
- Finally, the excited state returns to the ground state to cause chemiluminescence.
- Employing a co-reactant is especially useful when either radical charged ions are not stable enough for the ECL annihilation reaction, or radical ions cannot both be formed because of the solvent has a narrow potential window.
- With a co-reactant ECL can be generated by applying a potential in one direction.
- There are two reaction paths to produce the excited state of the ECL emitter, reductive-oxidation or oxidative-reduction ECL.
- For instance, oxalate ion ($C_2O_4^{2-}$) [4, 5] and several amines [6, 7, 8, 9] can be used for oxidative-reduction ECL where an oxidative step produces a strong reductant, whereas peroxidisulfate ion ($S_2O_8^{2-}$) is frequently used for reductive-oxidation ECL.
- As described above, ECL reactions require a luminophore.

- Few compounds and their derivatives primarily utilized for aqueous-based ECL bioanalytical detection methods, are luminol (5-amino-2, 3-dihydrophthalazine-1, 4-Dione) and ruthenium (II) chelates $[RuL_3]^{2+}$.
- Nowadays, new luminophores such as semiconductor nanomaterials are being widely used with great results. This fact is one of the main reasons ECL sensor and biosensor are having a great and successful advance.

2.3.4 Surface Plasmon Resonance Biosensor

- Surface Plasmon Resonance is a phenomenon that occurs when polarized light hits a metal film at the interface of media with different refractive indices.
- SPR techniques excite and detect collective oscillations of free electrons (known as surface plasmons) via the Kretschmann configuration, in which light is focused onto a metal film through a glass prism and the subsequent reflection is detected (Fig. 1).

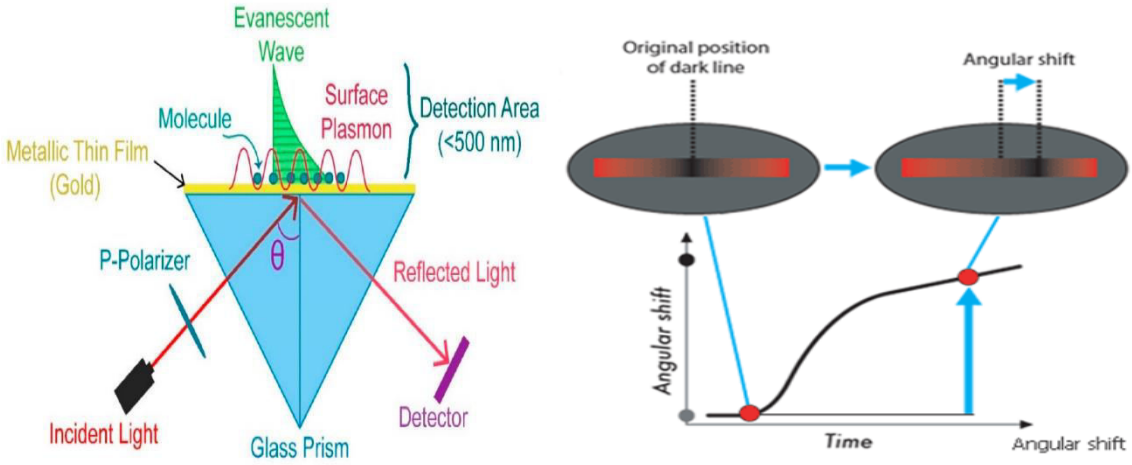


Fig 29: Basics of Surface Plasmon Resonance

- At a certain incident angle (or resonance angle), the plasmons are set to resonate with light, resulting in absorption of light at that angle. This creates a dark line in the reflected beam that contains a wealth of information.
- The resonance angle can be obtained by observing a dip in SPR reflection intensity.
- Comparing to the conventional diagnostic tools, SPR biosensors have multiple advantages such as easy preparation, no requirement of labelling, real-time detection capability, cost- effectiveness, and high specificity and sensitivity.
- However, for the label-free detection of low concentrations of analytes with small molecular weight its sensitivity is not enough.
- Therefore, considerable efforts have been invested to overcome these challenges and improve the sensitivity of the SPR biosensor such as ligands or functional nanomaterials with keeping all its advantages.

- The enhancement of the SPR biosensor needs modification of its surface with suitable ligands to capture the target compound (the analyte) and neglect other molecules available in the sample as shown in Figure 28.

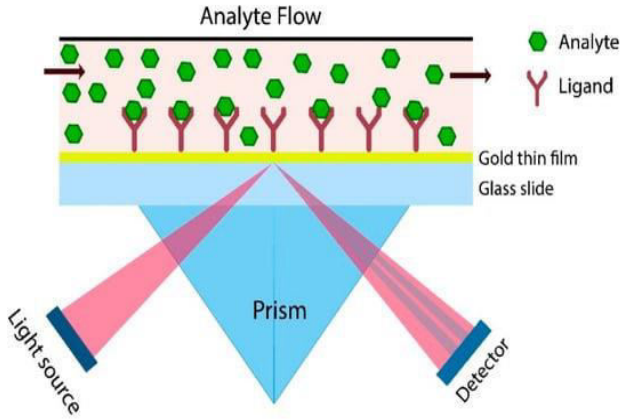


Figure 30. Direct label free detection SPR biosensor.

- These ligands can be permanently or temporarily immobilized on the sensor surface.
- The analyte accumulation results in a RI change in the evanescent field detected.
- When the ligand captures the analyte, the measurable signal rises and this is called direct label free detection.
- Following in time the resonance angle or wave length shift at which the dip is observed produces the sensogram (Figure 29), then the amount of adsorbed species after injection of the original baseline buffer can be determined, and a study of the kinetics of the biomolecular interaction can be done.

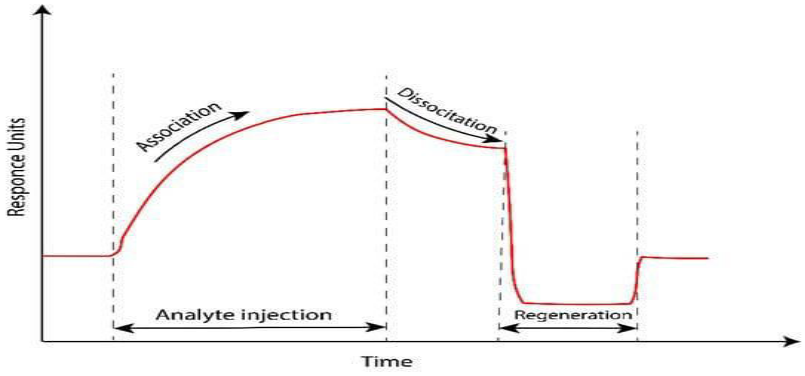


Figure 31. Surface plasmon resonance (SPR) sensogram.

2.3.5 Field-effect transistor (FET) biosensors

- Among various kinds of biosensors, field-effect transistor biosensors (Bio-FETs), an integrated between bio-receptors and ion-sensitive field-effect transistors (ISFET), emerged as the most developed candidates because of several advantages.

- In a typical FET system, the sensing elements are immobilized on the sensing channels (semiconductor path), which are connected to source (S) and drain (D) electrodes, to capture the targets (usually via high specificity and binding affinity).
- A bias potential is applied and modulated to a third electrode (gate).

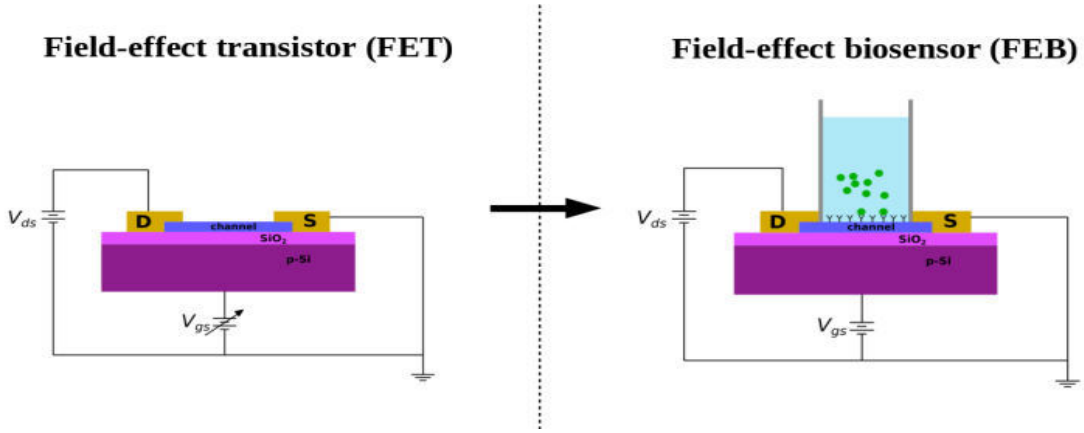


Figure 32. Field-effect transistor based biosensors.

- The channel conductance, which is varied by detection of the targets, is recorded and further processed by an electrical measurement system.
- There are two kinds of FETs: n-type with electrons as the main charge carriers and p-type with holes as the primary charge carriers.
- In an n-type FET system, if the probes detect positively charged molecules, the charge carriers (electrons) will accumulate on the sensing channels and increase the conductance.
- If negatively charged targets are recognized, the conductance will be decreased due to the depletion of the electrons.
- Conversely, for a p-type FET system, binding with positive charges results in conductance decline due to a reduction of the charge carriers (holes) and capturing negative charges raises the conductance because of hole accumulation.
- Outburst of nanotechnology triggers combination between biosensors and nanomaterials for sensing application with breakthrough designs in which biomolecules (antibodies, nucleotides and so on) as receptors are immobilized on the surface of nanotransducers (nanowires, nanotubes, nanoparticles, etc.).

2.3.6 Impedimetric biosensors

- Label-free electrochemical biosensors include impedimetric biosensors which measure the impedance, i.e., the opposition presented to a current in an alternating current (AC) circuit when a voltage is applied.
- The impedance is a complex quantity, and a common graphical representation is the Nyquist plot.

- This is a frequency response plot, where the values of the real part are plotted on the x-axis and those of the imaginary part on the y-axis.
- An ideal Nyquist plot shows a semicircle resulting from the dominating, kinetically limited charge transfer through the electric double layer at the electrode.
- Binding of analyte molecules to the electrode will influence the charge transfer and, hence, result in a shift of the Nyquist plot.
- As a consequence, the opportunity for charge transfer is an additional requirement for sensing layers of impedance biosensors.
- At low frequencies, Nyquist plots may show straight lines with a slope of 45°. This is characteristic for diffusion limited processes and described by the Warburg impedance.
- Electrodes of impedance biosensors are typically made of gold.
- A well-established procedure for the introduction of functional groups on this material is to use suitably substituted thiols forming self-assembled monolayers (SAMs).
- Thiols with aliphatic hydrocarbon spacers of sufficient chain length lead to well-defined and stable SAMs of high density.
- The brush-like structure of such layers makes it possible to effectively reduce nonspecific protein adsorption on the underlying gold surface.
- However, such SAMs may result in insulating layers, hindering the charge transfer required for the transduction principle of impedimetric biosensors.
- The use of aromatic hydrocarbons featuring delocalized π-electrons would be more beneficial for charge transfer processes, but nonspecific protein adsorption in the subsequent measurements may increase because of a reduced density of the layer.
- Conductive polymers would offer an alternative, but are often linked with coating procedures more complex than wet chemistry.
- Thiolated single-strand DNA (ssDNA) oligomers, on the other hand, can be packed densely on the gold surface by wet chemistry methods similar to those of thiolated hydrocarbons.
- Coimmobilization of thiolated ssDNA with thiolated hydrocarbons may be recommended to improve the integrity of the brush-like structure.
- The negatively charged backbone of the DNA oligomers—resulting from the composition of alternating sugar (deoxyribose) and phosphate groups—promises lower initial impedance values and, hence, the possibility of charge transfer events.
- With impedance sensors, immobilized ssDNA or immobilized oligonucleotides has been used directly as probe for DNA or protein detection, respectively.

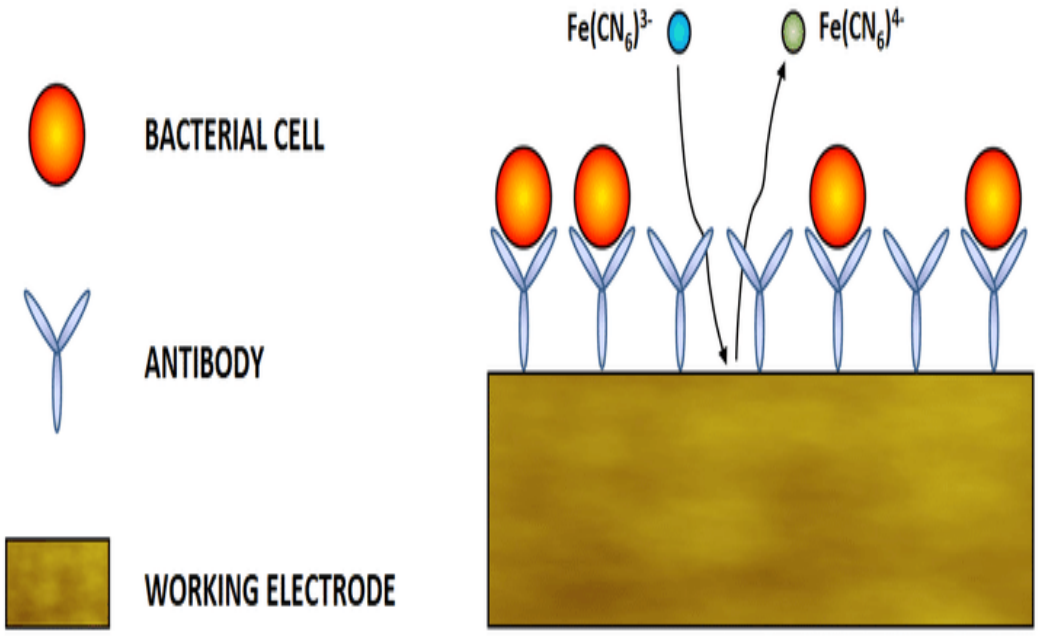


Fig 33 Impedimetric biosensors

2.3.7 Piezoelectric biosensor;

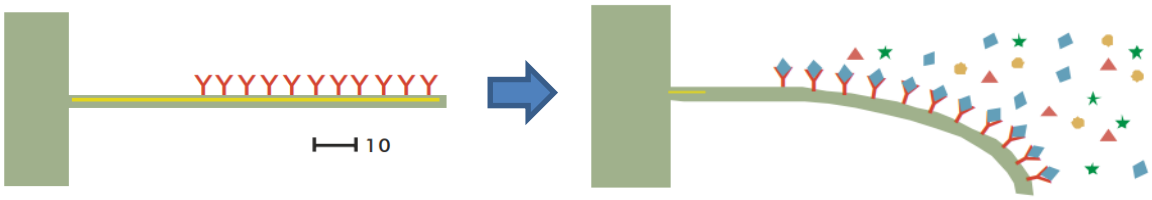
- Piezoelectric sensors utilize crystals which undergo an elastic deformation when an electrical potential is applied to them.
- The applied alternating potential produces a standing wave in the crystal at a characteristic frequency.
- This characteristic frequency is highly dependent on the elastic properties of the crystal.
- If a crystal is coated with a biological recognition element the binding of a (large) target analyte to a receptor will produce a change in the resonance frequency, giving a binding signal.
- In a mode that uses surface waves (SAW), the sensitivity is greatly increased.
- This is a special application of the Quartz crystal microbalance in biosensor.
- The piezoelectric generator is the method of transforming high frequency electric oscillations into mechanical oscillations.
- It is most frequently used at the present day for producing ultrasonic and it allows us to attain the highest frequencies now possible.
- Two classes of bio-recognition processes-bio-affinity recognition-and bio-metabolic recognition, offer different methods of detection.
- Both processes involve the binding of a chemical species with another, which has a complementary structure.
- This is referred to as shape-specific binding. In bio-affinity recognition, the binding is very strong, and the transducer detects the presence of the bound receptor-analyte pair.

- The most common types of processes are receptor-ligand and antibody-antigen binding.
- In bio-metabolic recognition, the analyte and other co-reactants are chemically altered to form the product molecules.
- The biomaterials that can be recognized by the bio-recognition elements are as varied as the different reactions that occur in biological systems.
- Almost all types of biological reactions, (chemical or affinity), can be exploited for biosensors. The concept of shape-specific recognition is commonly used to explain the high sensitivity and selectivity of biological molecules, especially antigen-antibody systems.
- The analyte molecule has a complementary structure to the antibody, and the bound pair is in a lower energy state than the two separate molecules. . The interaction of antibodies with their corresponding antigens is an attractive reason for attempting to develop antibody-based chemical biosensors, i.e. immunosensors

Cantilever Biosensor

- Cantilevers (springboard) are nanomechanical biosensors, microfabricated with the standard silicon technology.
- Due to their intrinsic flexibility, together with the availability of techniques designed to monitor bending, cantilevers have become versatile tools.
- This technology is a multifunctional and highly sensitive technique, and a real time method useful for a variety of applications, such as plastic explosive detection using gas biosensors, whole microorganism detection as part of liquid biosensors, or DNA and proteins studies.
- By incorporating a piezoresistor to each cantilever in a Wheatstone bridge type configuration, it is possible to read resistance changes as voltage changes.
- The Wheatstone bridge configuration uses a pair of cantilevers; one of them will be used as reference.
- The differential signal between both cantilevers will be the output of this configuration.
- The signal-noise relation is substantially improved with this configuration, and the noise originated by unspecific binding, thermal fluctuation, or vibrations is eliminated.
- Non-specific binding to the surface is a general problem that must be minimized in all analyses.
- Although the complete elimination of this parameter is not possible, its influence on detection could be controlled with the use of the reference cantilever.
- The immobilization of molecules on the cantilever surface is required for its use as a nanomechanical sensor (Figures 32).
- The immobilized molecules provide the cantilever with specificity for the analyte.

- The specific molecular interactions taking place at the flexible surface of cantilever increase surface tension, forcing the cantilever to bend.
- This type of surface tension induced by molecular interactions is not generally observed on the surface of common materials.
- The cantilever senses the tension and bends in response to the free energy changes taking place at its surface.



Figures 34 Nanomechanical cantilever sensor

Module 3

APPLICATIONS OF BIOSENSORS IN HEALTH AND ENVIRONMENT

3.1 Biosensors and diabetes management:

- Several factors have combined over the past few years to make glucose biosensor one of the most financially attractive areas in medical diagnosis.
- Generally, glucose measurements are based on interactions with one of three enzymes: hexokinase, glucose oxidase (GOx) or glucose-1-dehydrogenase (GDH).
- The hexokinase assay is the reference method for measuring glucose using spectrophotometry in many clinical laboratories.
- Successful glucose biosensor must also meet the diabetic's expectations.
- There are many theories of operations of biosensors such as Reflectance based method, Electrochemical methods, Enzymes and reagents, Hexokinase methods, Glucose oxidase based- Peroxidase optical method, Organic mediator optical methods, Perssian Blue method, Ferricyanide electrochemical method, Ferrocene Electrochemical method, reusable sensors etc.
- Electrochemical sensors may be subdivided into potentiometric, amperometric, or conductometric types.
- Enzymatic amperometric glucose biosensors are the most common devices commercially available, and have been widely studied over the last few decades.
- Amperometric sensors monitor currents generated when electrons are exchanged either directly or indirectly between a biological system and an electrode

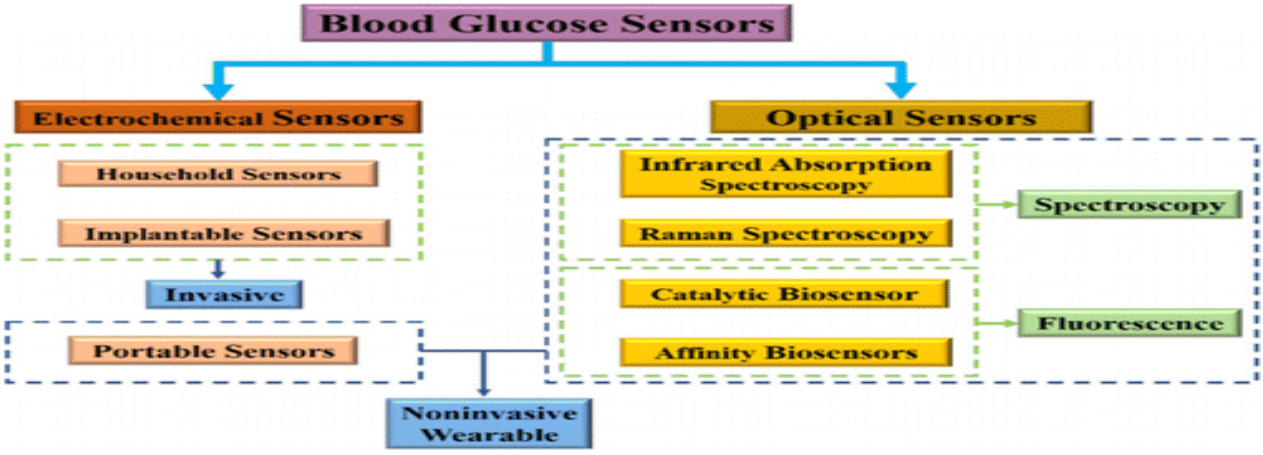
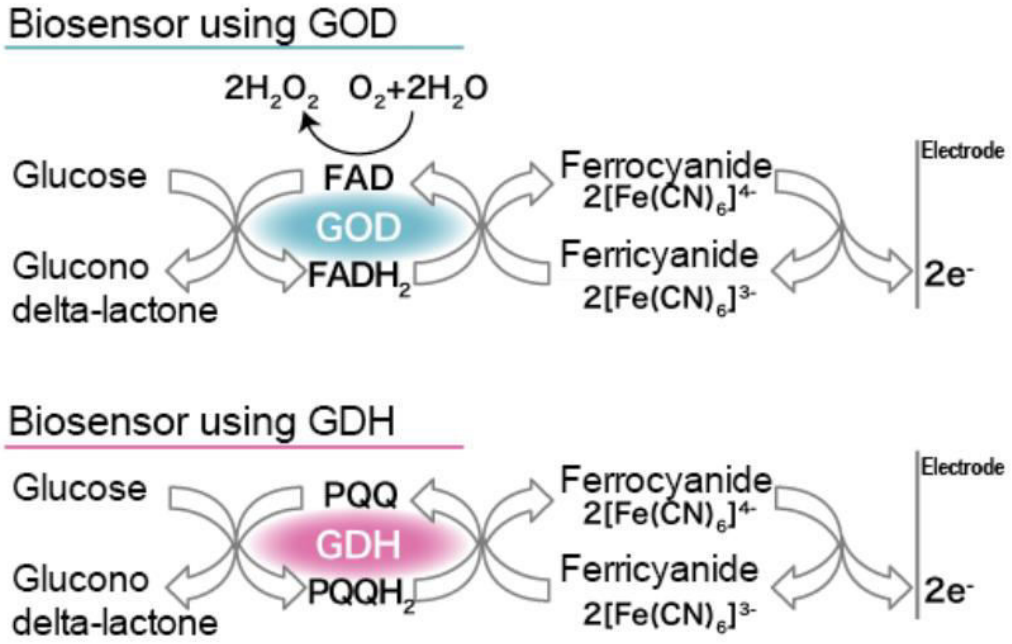


Fig. 35. Classification of blood glucose sensors in this review.

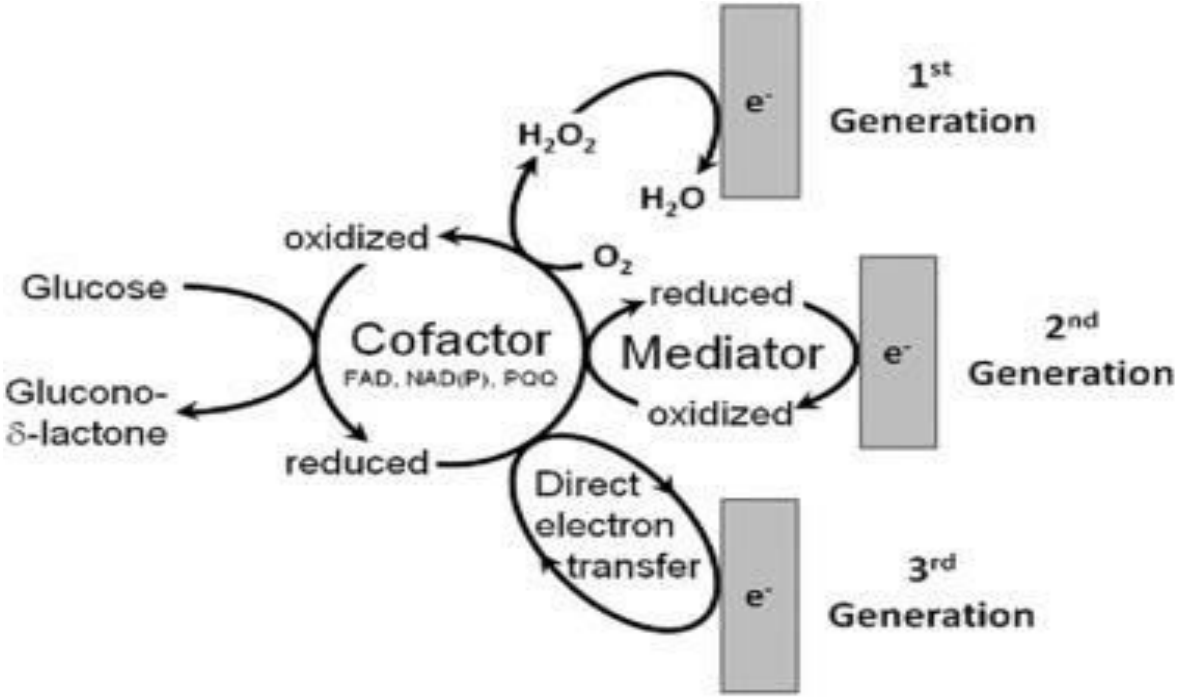
- Electrochemical methods are popular, and over the years, enzymatic amperometric glucose sensors were the first and widespread glucose sensors available
- Electrochemical glucose sensors can be divided into potentiometric (employed to detect variations of surface charge onto a counter electrode), amperometric (charge flow between the counter electrode and the bio-system), or conductometric (variations in ionic conductance between electrodes.
- They are generally fabricated by using two families of enzymes, the glucose oxidase and the glucose dehydrogenase (GDH). The reaction, catalyzed is as follows:



Figures 36 Glucose sensor reaction scheme

- GOx is the standard enzyme for biosensors; it has a relatively higher selectivity for glucose. GOx is easy to obtain, cheap, and can withstand greater extremes of pH, ionic strength, and temperature than many other enzymes, thus allowing less stringent conditions during the manufacturing process and relatively relaxed storage norms for use by lay biosensor users
- The concept behind a glucose biosensor is based on the fact that the immobilized GOx catalyzes the oxidation of D-glucose by molecular oxygen producing gluconic acid and hydrogen peroxide.
- To function as a catalyst, GOx requires a flavin adenine dinucleotide (FAD) redox cofactor. FAD functions as an initial electron acceptor and is reduced to FADH2.
- This enzymatic electrode evaluates the glucose level by the amperometric tracking of the released hydrogen peroxide.

- Glucose dehydrogenases are instead defined as oxidoreductases which are unable to use oxygen as an electron acceptor and therefore transfer electrons to other natural and artificial acceptors.
- GDHs also need a cofactor. These are mainly nicotine adenine dinucleotide (NAD+ or NADH depending on the oxidation state) or pyrroloquinoline quinone (PQQ).
- FAD, NAD+ and PQQ remove hydrogen, H+ and e-, from glucose.
- GDH-PQQ is a particularly efficient enzyme system, with a fast electron transfer rate, but it is relatively expensive.
- GDH with NAD+ as a cofactor produces NADH rather than H_2O_2.
- Nicotine adenine dinucleotide is an important electron acceptor in glucose oxidation, during which NAD's nicotinamide ring accepts one hydrogen ion and two electrons, equivalent to one hydride ion.
- In this reaction, the generated reduced form of this cofactor is NADH, which can be electrochemically oxidized.



Figures 37 Glucose sensor designs

- 1st Generation: Based on the sensor designed by Clark and Lyons
  Formation of hydrogen peroxide
  Oxygen as an electron acceptor
  Errors due to interference from other electroactive species
- 2nd Generation Replacement of oxygen as an electron acceptor
  Introduction of the non-physiological mediator

	Limitations in the transfer from the enzymatic active site to the electrode
3rd Generation	Absence of mediator
	Direct transfer between enzyme and electrode
	Low operating potential, higher selectivity, less interference

- An electrochemical biosensor is composed by working electrodes (on which the reaction of interest, responsible for the measurement, takes place), reference electrodes and auxiliary electrodes (to ensure that the current does not circulate through the electrode).
- Glucose concentration is mostly evaluated using the amperometric method that monitors the current flowing between the working electrode and the reference electrode.
- The basic concept of the glucose biosensor is based on the fact that the immobilized GOx catalyzes the oxidation of β-D-glucose by molecular oxygen producing gluconic acid and hydrogen peroxide. In order to work as a catalyst, GOx requires a redox cofactor—flavin adenine dinucleotide (FAD). FAD works as the initial electron acceptor and is reduced to FADH2.

$$\text{Glucose} + \text{GOx} - \text{FAD}^{+} \rightarrow \text{Glucolactone} + \text{GOx} - \text{FADH}_2$$

- The cofactor is regenerated by reacting with oxygen, leading to the formation of hydrogen peroxides.

$$\text{GOx} - \text{FADH}_2 + \mathbf{O}_2 \rightarrow \text{GOx} - \text{FAD} + \mathbf{H}_2\,\mathbf{O}_2$$

- Hydrogen peroxide is oxidized at a catalytic, classically platinum (Pt) anode. The electrode easily recognizes the number of electron transfers, and this electron flow is proportional to the number of glucose molecules present in blood [36].

$$H_2O_2 \rightarrow 2H^{+} + O_2 + 2e$$

- Three general strategies are used for the electrochemical sensing of glucose; by measuring oxygen consumption, by measuring the amount of hydrogen peroxide produced by the enzyme reaction or by using a diffusible or immobilized mediator to transfer the electrons from the GOx to the electrode.
- The number and types of GDH-based amperometric biosensors have been increasing recently. The GDH family includes GDH-pyrroquinolinequinone (PQQ) and GDH-nicotinamide-adenine dinucleotide (NAD). The enzymatic reaction of GDH is independent of the dissolved oxygen. The quinoprotein GDH recognition element uses PQQ as a cofactor.

$$\text{Glucose} + \text{PQQ(ox)} \rightarrow \text{gluconolactone} + \text{PQQ(red)}$$

- This mechanism requires neither oxygen nor NAD^{+}. GDH-PQQ is a particularly efficient enzyme system, with a rapid electron transfer rate, but it is relatively expensive [17].

- GDH with NAD as a cofactor produces NADH rather than H_2O_2. NAD is a major electron acceptor in the oxidation of glucose, during which the nicotinamide ring of NAD^+ accepts a hydrogen ion and two electrons, equivalent to a hydride ion. The reduced form of this carrier generated in this reaction is called NADH, which can be electrochemically oxidized.

$$\text{Glucose+NAD+}\rightarrow\text{gluconolactone+NADHNADH}\rightarrow\text{NAD++H++2e}$$

3.2 Microfabricated biosensors and point-of-care diagnostics systems

- Even with tremendous growth in hospital sector, providing centralized clinical testing facility is inadequate.
- Patient admitted to the hospital with number of possible life-threatening causes for symptoms during initial assessment may get unattended in initial and comprehensive qualitative and quantitative assessment to get information that is crucial in managing the disease by any doctor.
- Without clinical chemistry or microbiological parameters physician is often unable to make any reliable diagnosis.
- To do this clinical samples need to be draw from the patient, labelled, transported to laboratory, segregated, tested, analysed, certified and reported back that often located at central place that takes several hours due to remote locations of the laboratory and its expensive equipment.
- So, such delay is major concern as physician is unable to initiate timely effective treatment.
- Hence, approach to solve such issues can only be solved by addressing laboratory-based steps, and not through transportation logistic problem.
- However, approached in delaying the testing time by automation of the analysis lead to increase in the coast of testing due to expensive instruments.
- Hence, "Point-of-Care" clinical sample analysis system incorporates simple, safe, maintenance-free home-use monitoring system works with whole clinical samples rather than clinical samples that requires preclinical treatments, so precludes all the laboratory instruments such as centrifuges, filters etc. that requires money and time.
- Such "Point-of-Care" blood analysis system should requires design that (a) replace in-line calibration with factory calibration, (b) replace wash step with single-use analytical component, (c) eliminate need for metered samples, (d) simple reusable reading device to display results.

- "Point-of-Care system" that can be utilised along the patient's "bed-side" rather than the laboratory's "table-top" with the same analytical quality, along emergency room, intensive care unit, critical care unit etc.
- "Point-of-Care system" is crucial for estimating clinical parameters such as blood gases, electrolytes, metabolites that covers many conditions such as arrhythmia, dehydration, anemoia, respiratory problems, and diabetes.
- A successful hospital "Point-of-Care system" would, at minimum, need to perform all of these.
- The microfabrication technology focuses on the miniaturization of engineering systems and has evolved from the mature process technology in semiconductor device fabrication.
- The main techniques of microfabrication are thin films deposition, layers doping, patterning via photolithography, etching to obtain the required design, polishing and bonding.
- Microfabrication technology has been widely used for the development of complex electronic components, integrated micro-electromechanical systems (MEMS), and different types of sensors for a wide range of applications.
- The benefits of developing sensors with such technological processes include low costs, large scale fabrication of nominally identical structures, the possibility of integration with other devices and the compatibility with a numerous of technologies, such as complimentary metal-oxide semiconductor technology (CMOS) for the manufacturing of integrated circuits or silicon on insulator (SOI) technology for semiconductor engineering.
- The most common assembly of electrodes for electrochemical applications is the three-electrode system.
- A three-electrode structure consists in a working electrode (WE), a reference electrode (RE) and a counter electrode (CE).
- The working electrode serves as the transduction element in the (bio) chemical reaction, while the counter electrode establishes a connection to the electrolyte solution so that a current can be applied to the working electrode.
- This system is also beneficial because it averts the RE from pushing the current which could modify its potential.
- The potential is applied between the WE and the RE and the CE provides the mandatory current to sustain electrolysis at the WE.

Fundamental of POC biosensors

- Biosensors are analytical devices used for the detection of a biological substance. In general, biosensors consist of three components: a receptor (specific for a disease) that recognizes the

analyte, a transducer that converts the bio-recognition event into a measurable signal, and a reader.

- This technology allows precise control and manipulation of fluids, which typically requires much less sample volume than that of conventional assays.
- The efficient liquid mixing in biosensors also enhances the interaction between assay reagents and target biomarkers, which shortens the assay duration and provides fast readout.
- Moreover, the portability of biosensors makes them ideal candidates for POC field settings. To date, researchers around the globe have developed various types of biosensors for wide applications in POC settings such as the diagnosis of infectious diseases, food safety analysis, and environmental monitoring.
- These biosensors include chip-based, paper-based, and other biosensors (textile-based or nanomaterial-based biosensors), which will be briefly discussed in the following sections.
- For POC testing of chronic and infectious diseases, there is always an increasing demand for low-cost, portable, and integrated biosensors, which can provide rapid results with low sample consumption.
- Chip-based biosensors are one of the POC biosensors used for POC diagnosis of many infectious diseases.
- They are prepared either by miniaturizing conventional biochemical assays on a microchip scale or by integrating novel detection principles with microfluidic chips, referred as "Lab-on-chip".
- These biosensors are mainly made of polymethyl methacrylate (PMMA), polytetrafluoroethylene (PTEE), or polydimethylsiloxane (PDMS).
- PDMS biosensors are commonly used due to their cost-effectiveness, high specificity, and minimal reagent consumption.
- Specifically, they consist of multiple channels which enable nucleic acid testing steps, including nucleic acid extraction, amplification, and amplicon detection, to be performed in an automated manner.
- In recent years, smartphone has also been integrated into chip-based biosensors for imaging and signal analysis.
- For instance, chip-based biosensors coupled with a smartphone have been used to rapidly detect amplicon signals within an hour for the diagnosis of H1N1 and Zika virus infections.
- Paper-based biosensors have been broadly used for rapid testing of infectious diseases, which show potential to substitute the conventional laboratory tests and chip-based biosensors.

- Paper is inexpensive, readily available, and biodegradable, showing a promising tool for onsite rapid diagnosis.
- It allows the diffusion of a biological sample through a capillary effect, eliminating the need for external power sources.
- Earlier studies have introduced lateral flow test strips and microfluidic paper-based analytical devices (μPAD) for POC testing.
- The assay usually involves hybridization of single-stranded DNA or RNA with a complementary probe to produce double-stranded nucleic acids or interaction between antigen and antibody to produce an Ag-Ab complex, generating signals such as colorimetric, fluorescence, or chemiluminescence signals.
- Their special characteristics such as simple, affordable, and ease of fabrication, modification, and functionalization have made them possible to achieve rapid, onsite POC testing.
- Other biosensors like film-based, textile-based, and nanomaterial-based biosensors have also been used for the diagnosis of infectious diseases.
- For instance, film-based biosensors which are made of transparent polyester substrate film have been used for the detection of pathogens.
- This material has the ability to withstand thermal cycling and amplification process.
- Textile-based biosensors are biosensors which are typically made of thread, fabric, or clothes which are inexpensive and readily available with low sample consumption. For example, textile-based biosensors are cheaper and require a smaller sample volume (optical
- Emerging point-of-care biosensors for rapid diagnosis of COVID-19 have exploited these biosensors for POC diagnoses, especially by integrating with mobile phones.
- The device packaged in a compact portable assembly shows a bright potential for performing fast and accurate cytokine assays for COVID-19 in clinics and POC settings for responsive disease management.

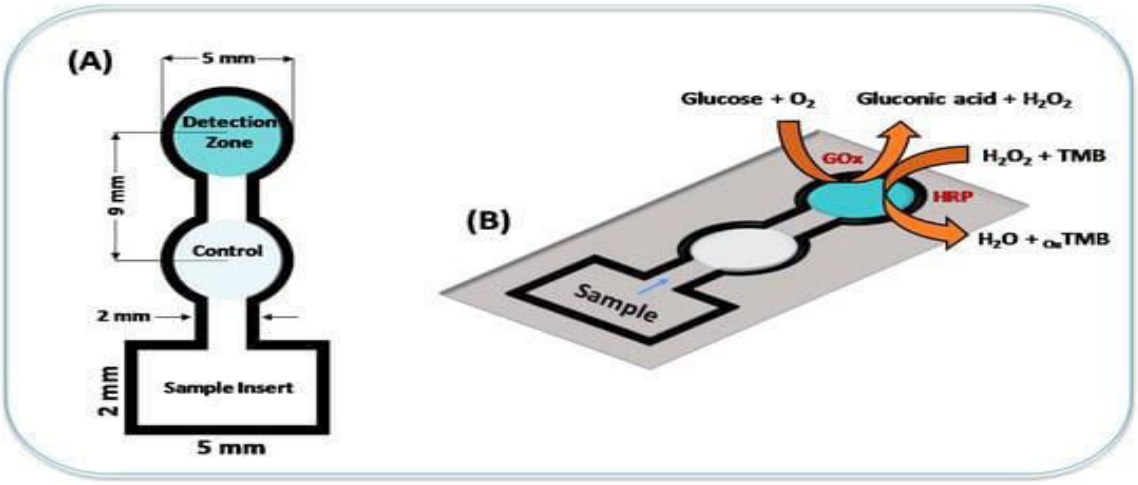


Figure 38. Presentation of (A) the layout of MFBS used for glucose colorimetric assays and (B) a simplified view of the enzymatic reaction involved in the presence of chromogenic reagent (TMB) for glucose detection.

3.4 Noninvasive biosensors in clinical analysis:

- Neonatal, elderly and diabetic patient who have to undergo daily metabolite measurement have problem in giving samples demand non-invasive method,
- Noninvasive method does not require invasion of the body to collect sample, as these method can efficiently detect metabolites directly in urine, swat, saliva, or skin.

3.4.1 Non Invasive Glucose Monitoring

- Multitude principles and techniques of non-invasive glucose monitoring have been pursued in academic research as well as in industry in recent times.
- Among them, broadly four particular non-invasing glucose monitoring principles are widely investigated and reported.
- These four principles are differentiated based on the principles of detection of glucose. They are as follows:
    - Optical Spectroscopy (Optical Detection)
    - Photoacoustic Spectroscopy (Acoustic Detection)
    - Electromagnetic Sensing (Electromagnetic Detection)
    - Nanomaterial Based Sensing (Electrochemical Detection)
- **Optical spectroscopy:** The detection principle of the Optical Spectroscopy is based on the fact that glucose influences the optical signal passing through it by absorption of light at some particular overtones and combination band wavelengths in the mid infrared (mid-IR) and nearinfrared (NIR) spectrum regions.

- **Photoacoustic Spectroscopy:** The photoacoustic glucose monitoring is a hybrid approach, which combines optical excitation and acoustic detection in determining the glucose concentration. Here, the optical energy from the excitation is converted into an acoustic energy by a multistage energy conversion process.
- **Electromagnetic sensing:** Human blood consists of 55% of plasma. The blood plasma consists of around 90% of water. Water being a dipolar compound, the polarization is high. This makes the relative permittivity to be high as well. In contrast, glucose has a smaller relative permittivity, being less polarized. Thus, the overall increase or decrease in glucose concentration in the same volume of blood sample reduces or elevates the relative permittivity of blood plasma, respectively. The measure of the relative permittivity by multiple techniques of electromagnetic sensing at a particular microwave/mm-wave frequency gives the value of the glucose concentration in blood in the form of measured electrical power in a very effective manner which helps in the measurement of blood glucose. It is important to note that the dielectric properties of blood are modified because the change in blood glucose levels is of much greater extent than due to changes of other compounds present in the blood.
- **Nanomaterial based sensing**: The emergence of nanomaterials, starting from metallic gold, silver, copper oxide, iron oxide to polymer composites, carbon nanotubes and graphene, as primary components in sensing technologies, has significantly upgraded the modern-day biosensors, extracting valuable physiological data and useful information from essential body fluids like urine, saliva, sweat and tears. The impact of nanomaterials in sensing applications is remarkable as they exhibit large surface area, enhanced sensitivity and selectivity, improved catalytic activities which are essential prerequisites for an accurate and precise estimation of glucose levels in humans.

3.4.2 Alcohol based sensor.

- Measurement of alcohol in saliva is one of the frequently performed test in legal case to establish the degree of alcohol intoxication, which is one of the major cause of traffic accidents.
- Impairment of driving ability starts as low as 50 mg of ethanol per 100 mL of blood.
- Measurement of ethanol in saliva, urine and blood are of great practical importance in forensic purpose.
- Measurement of ethanol is used in industry to estimate the levels of ethanol produced during fermentation.
- Saliva alcohol device requires a specific, sensitive, reliable alcohol sensor,
- The two most appropriate base sensor for alcohol electrode are oxygen electrode and hydrogen peroxide electrode

- When these sensors are coupled with alcohol oxidase, response to alcohol is achieved based on reaction.

$$\text{Alcohol} + O_2 \xrightarrow[\text{Alcohol oxidase}]{} H_2O_2 + \text{Acetaldehyde}$$

- Assay of the alcohol is achieved by measuring the decrease in oxygen with an oxygen electrode or by measuring the increase in hydrogen peroxide with hydrogen peroxide electrode during initial rate of reaction of 12 s or steady state current at 1-2 min after injection of ethanol.

(Refer Amperometic biosensor principle in Module 1, Page 11).

3.4.3 Lactate based sensor

- Lactate is an important metabolite that need to be monitored in critical care patients, diabetic control, food analysis and orts medicine.
- Electrochemical Amperometric acetate probe operates in following way.

$$\text{Lactate} + O_2 + H_2O \xrightarrow[\text{Lactate oxidase}]{} \text{Pyruvate} + \text{Acetaldehyde}$$

- Assay of the lactate is achieved by measuring the increase in hydrogen peroxide with platinum working electrode and silver/silver chloride reference-counter electrode. Current change due to oxidation of hydrogen peroxide is proportional to the lactate concentration in the sample.

(Refer Amperometic biosensor principle in Module 1, Page 11).

3.4.4 Transcutaneous arterial oxygen tension sensor

- A Clark amperometric electrode is used in a sensor unit that is placed in contact with the skin.
- Three Glass- glass sealed platinum cathode separately connected via current amplifier to an Ag/Agcl anode ring, and are dipped in KCl electrolyte where chemical reaction takes place.
- Under normal condition partial pressure of oxygen on skin surface is that of atmospheric regardless of the pressure beneath the skin.
- Hyperemia of the skin causes partial pressure of oxygen to approach to that of arterial partial pressure of oxygen, when induced by drugs administration, applying nicotinic acid, heating or abrasion of the skin during measurement.
- Heating elements are integrated in the sensor with thermistor sensor to heat the skin between 43-44^{o}C.
- Oxygen permeable membrane separates the inner electrodes and outer environment.

(Refer Amperometic biosensor(Oxygen Electrode) principle in Module 1, Page 11).

3.4.5 Transcutaneous arterial carbon dioxide tension sensor

- Transcutaneous arterial carbon dioxide tension sensor is similar to transcutaneous arterial oxygen tension sensor except that sensor is potentiometric sensor.

- Glass pH electrode with concentric Ag/AgCl reference electrode.
- Concentric Heating elements are integrated in the sensor with thermistor sensor to heat the skin between 43-44^{o}C.
- Carbon dioxide permeable Teflon membrane separates the inner electrodes and outer environment.

(Refer Potentiometric biosensor (pH electrode based) principle in page number 13)

3.5 Surface plasmon resonance and evanescent wave biosensors

- Surface plasmon resonance (SPR) is a unique optical surface sensing technique with numerous applications in variety of discipline.
- SPR can be used to probe refractive index changes that occur within the vicinity of a sensor surface.
- Thus any physical phenomenon at the surface that alters the refractive index will elicit a response.
- The basic SPR apparatus is generally referred to as the Kretschmann prism arrangement.
- Thin film of metal is coated on one face of a prism, or alternatively, the metal film can be deposited onto a glass slide that is brought into optical contact with the prism using refractive index matching fluid.
- This metal film forms the sensor surface on which the bio system sample is placed.
- Light is launched into prism, where it is both coupled into the plasma mode of the metal film as well as being partially reflected off the metal film to an optical photometer.
- The changes in the amount of light striking the detector represent the sensor output.
- This output is affected by changes in the bio system layer on the metal film on the metal film, which in turn affects the refractive index of the metal film-bio system pair, which in turn affects amount of light that couples that coupled into the surface plasmon mode of the metal film.
- Evanescent wave sensor concepts is composed of three major concepts, a bilayer on surface which specifically and directly or indirectly binds the analyte of interest, and a transducer which forms a binding event between the bilayer and the analyte into an electric signal that serves as a quantifiable output for simple analysis.
- This is generally achieved by immobilizing one of the receptors on the surface of the transducer.
- For direct assay binding event itself is detected, and since this event does not provide significant signals, physical even is detected via changes in local density, dielctic constant or refractive index using electrical, gravimetric, and optical transducers.

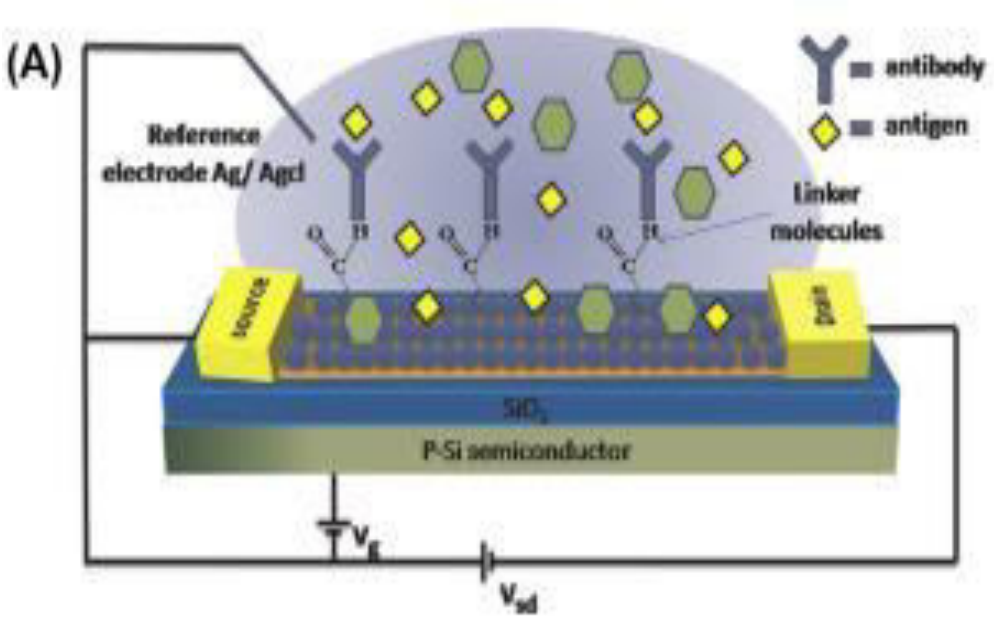


Fig 39. Basic components of a surface plasmon resonance (SPR) system

- SPR-based biosensors demonstrate interesting potential for environmental applications, and a substantial number of studies have been reported for heavy metals, dioxins, pesticides, aromatic hydrocarbons, phenol, polychlorinated biphenyls and detection.
- Biosensors can be used to monitor environmental pollution in air, soil, water, etc.; toxic elements in food and quality control; biohazardous bacteria or virus, and biomolecules for clinical diagnostics, etc.; and to necessitate sensitive, fast, and selective equipments or tools.

3.6 Biosensor in cancer and HIV early diagnosis.

- The timely diagnosis of cancer represents the best chance to increase treatment success and To reduce cancer deaths.
- Nanomaterials-based biosensors containing graphene quantum dots (GQDs) as a sensing platform show great promise in the early and sensitive detection of cancer biomarkers, due to their unique chemical and physical properties, large surface area and ease of functionalization with different biomolecules able to recognize relevant cancer biomarkers.
- The developed optical, electrochemical and chemiluminescent biosensors based on GQDs have been shown to ensure the effective diagnosis of several cancer diseases as well as the possibility to evaluate the effectiveness of anticancer therapy.
- The wide linear range of detection and low detection limits recorded for most of the reported biosensors highlight their great potential in clinics for the diagnosis and management of cancer.

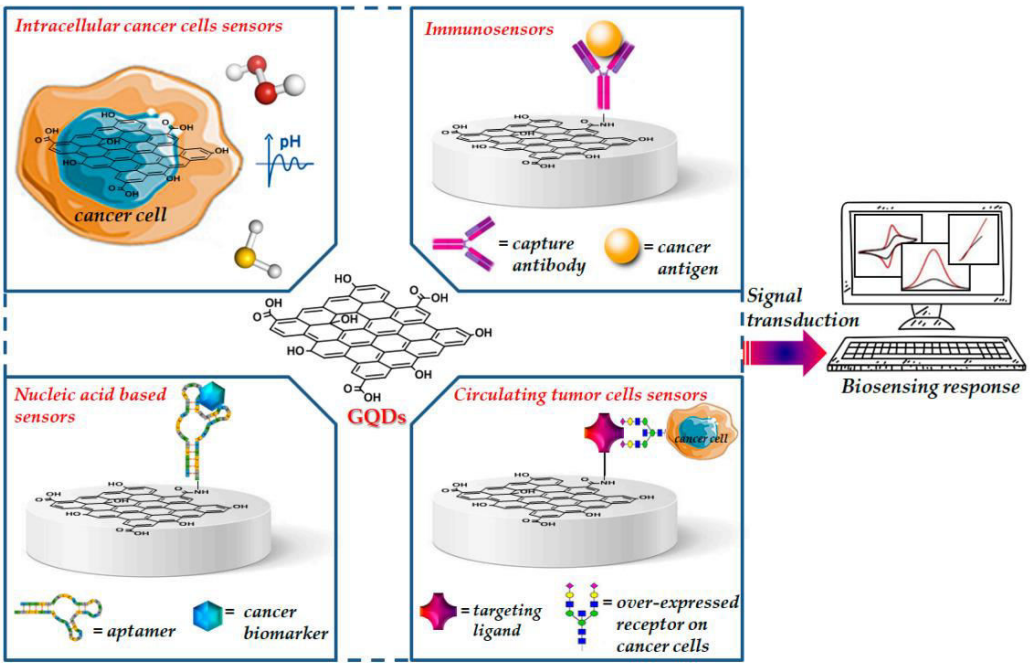


Fig 40 Representation of Graphene quantum dots for cancer detection

- GQD is new class of fluorescent materials from carbon nanomaterials family, possess ideal chemical and physical properties to be used and integrated in sensors for biological and medical applications.
- From a morphological point of view, GQDs show the peculiar features of both graphene and carbon dots (CDs).
- CDs and GQDs are zero dimensional carbon based materials, both endowed with unique physicochemical properties associated with quantum size and edge effects.
- While CDs are mainly synthesized by bottom-up strategies and show spherical shape up to 10 nm,
- GQDs are typically derived from materials where the sp2 carbon atoms are organized into a graphene structure.
- The presence of a graphene structure and the large surface to volume ratio allow the ease functionalization to a large number of sites of graphene surface.
- Antibodies, proteins, nucleic acids and polymers have been covalently linked and/or conjugated via π–π interaction to the graphene surface of GQDs, affording high selective and sensitive biosensors able to recognize biomarkers associated with various types of cancers.
- The electronic and optical properties of GQDs make these nanomaterials particularly attractive in optoelectronics.
- These properties depend on their band gap because of quantum confinement, and are influenced by their size, number of layers, shape and edge configuration.

- As a consequence, these properties can be modulated by using optimal carbon sources and size-controlling synthetic methodologies.
- Human immunodeficiency virus (HIV) is a global epidemic; however, many individuals are able to obtain treatment and manage their condition.
- Progression to acquired immunodeficiency syndrome (AIDS) occurs during late-stage HIV infection, which compromises the immune system, making it susceptible to infections. While there is no cure, antiretroviral therapy can be used provided that detection occurs, preferably during the early phase.
- However, the detection of HIV is expensive and resource-intensive when tested with conventional methods, such as flow cytometry, polymerase chain reaction (PCR), or enzyme-linked immunosorbent assays (ELISA).
- Improving disease detection in resource-constrained areas requires equipment that is affordable, portable, and can deliver rapid results.
- Microfluidic devices have transformed many bench top techniques to on-chip detection for portable and rapid point-of-care (POC) testing.
- These devices are cost-effective, sensitive, and rapid and can be used in areas lacking resources.
- Moreover, their functionality can rival their bench top counterparts, making them efficient for disease detection.

Chapter 4

Applications of Biosensors in Food and Agriculture Industry

- Detection of product content, allergic components, pathogens, pesticide residues.
- Monitoring of raw material conversions.
- Detection of crop diseases, pathogens in plants,
- Detection of soil nutrients, pesticide and its residual detection.

4.1 Detection of product content, allergic components, pathogens, pesticide residues.

Detection of food content

- Several changes can take place in packaged food as a result of metabolism or microbial growth over time.

  For example, changes in gas evolution or microbial accumulation can be used to obtain information about the status of food, e.g., freshness or degradation.
- Sensors that can measure such changes could provide an overall estimation of food quality. Examples include "on-package" pH indicators that change color when food decays as a result of pH changes associated with the release of volatile amines generated during meat or fish spoilage.
- Commercially available freshness indicators for different types of food including fish, meat, and poultry, cereal grains, fruits, and vegetables are as follows.

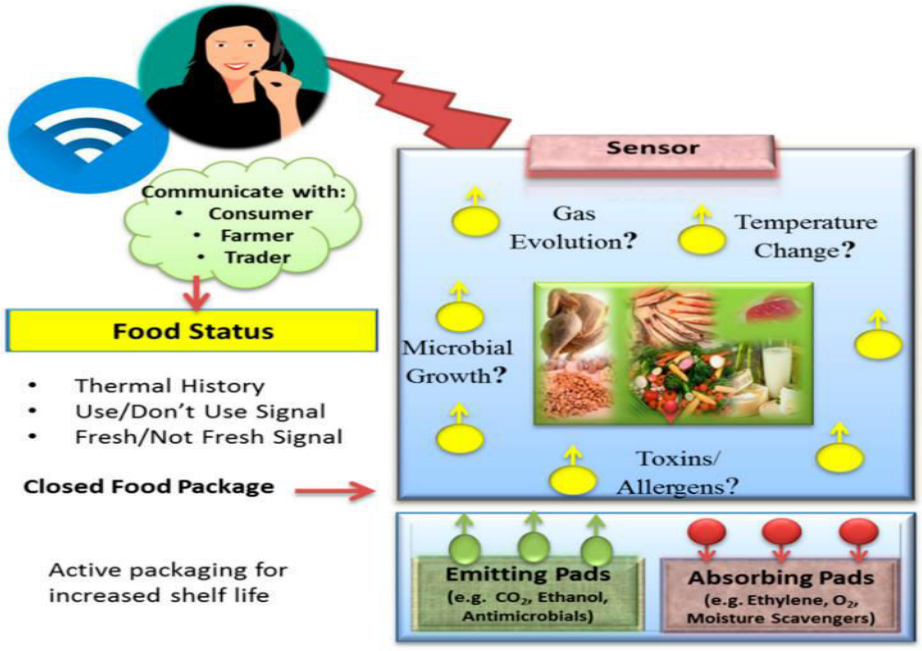


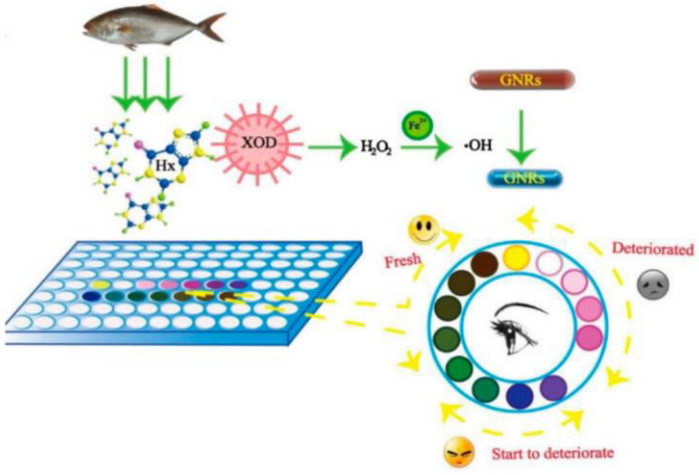
Fig 41. Representation of the concept of smart and active packaging technology

Fish, Meat, and Poultry

- When meat, fish, or poultry undergo degradation, different spoilage indicators can be found indicating lipid decay, protein breakdown, and adenosine triphosphate (ATP) decay.
- The speed of degradation is dependent on the type of product, storage temperature, feeding habits, and harvesting methods.
- Traditional methods to assess freshness rely on human senses; although they are essential, they provide no quantitative data of spoiled food.
- Methods that can quantitatively measure markers of degradation through chemical or biological reactions can provide the means to more precisely assess the status and quality of food.
- In fish products, for example, one of the main freshness indicators is hypoxanthine, which is produced by the metabolic degradation of ATP.

$$\text{Hypoxanthine} + O_2 \xrightarrow{\text{Xanthine oxidase}} \text{Xanthine} + H_2O_2$$

$$\text{Xanthine} + O_2 \xrightarrow{\text{Xanthine oxidase}} \text{Uric acid} + H_2O_2$$



Fog 42 colorimetric freshness sensor for fish via hypoxanthine detection

Cereal Grains

- One of the indicators of grain spoilage during storage is the emission of CO_2 as a result of insect infestations, and mold spoilage causing grain deterioration or the production of harmful mycotoxins.
- Developing CO_2 sensors for early spoilage detection has been reported.
- Sensor developed based on polyaniline boronic acid (PABA) conducting polymer for measuring CO_2 levels in the range of 380–2400 ppm in grain.

- The sensing mechanism is based on the conversion between the emeraldine salt form and the insulating emeraldine base form of polyaniline and PABA through protonation and deprotonation.
- When gaseous CO_2 reacts with water, it creates carbonic acid that protonates the polyaniline and further increases conductivity as CO_2 partial pressure increases.
- Gluten is another component of interest for grain analysis, as certain individuals can develop gluten intolerance that can cause serious disorders of the digestive system.
- The most common method for gluten analysis is by the conventional enzyme-linked immunosorbent assay (ELISA).
- Analysis of toxicity caused by mycotoxin contamination in cereals is also of special interest.
- Mycotoxins, such as o chratoxin A (OTA), aflatoxins, trichothecenes, fumonisins, zearalenone, and ergot alkaloids, are produced by fungi.
- Several biosensor types for mycotoxin detection have been reported such as electrochemical biosensors were developed for the detection of OTA using a competitive mechanism using OTA-specific aptamers and horseradish peroxidase (HRP) enzyme [70].

Fruits and Vegetables

- Technology for monitoring and preserving fruits and vegetables is necessary to decrease food loss during transportation and storage.
- Many fruits and vegetables produce ethylene due to environmental stress after being harvested.
- Ethylene can enhance ripening even at extremely low concentrations.
- The presence of aging fruits and vegetables close to fresh ones can also cause aging and ripening as ethylene is emitted.
- Ethylene can be removed by using ethylene absorbers or oxidizers (scavengers).
- Scavenging systems facilitate removal, thus lowering the loss of other products due to overproduced ethylene.
- The most available ethylene scavenger is potassium permanganate (KMnO4), which oxides ethylene to ethylene glycol and can be further oxidized to CO_2 and H_2O, producing dark brown MnO_2.
- Several commercial scavengers have been developed based on ethylene chemisorption by $KMnO_4$ granules over clays or activated carbon.
- Volatile organic compounds (VOC) accumulate in the presence of fruits and vegetables in closed containers or packages.

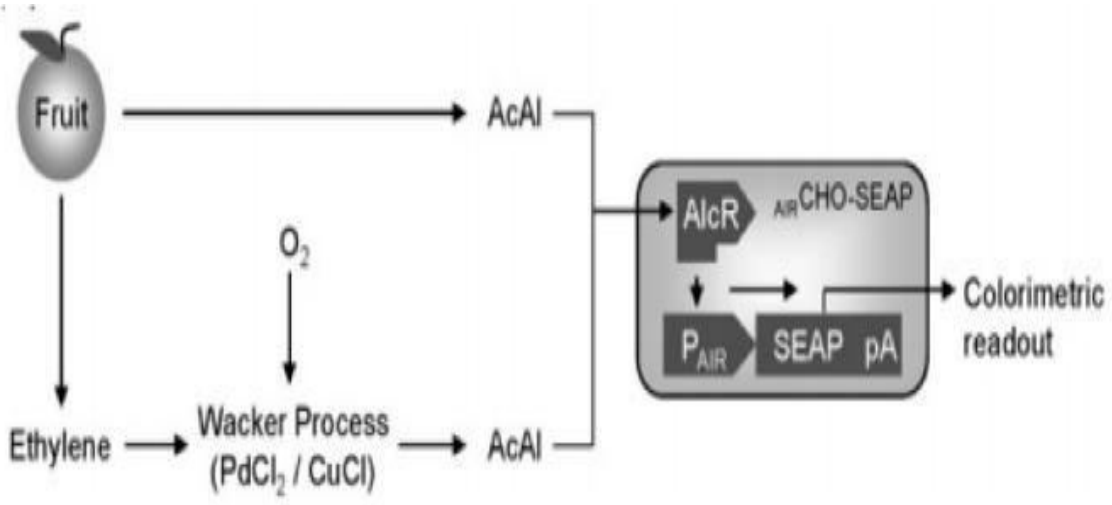


Fig 43: Dual-channel catalytic-biosensor. Acetaldehyde (AcAl) generated by fruit diffuses through the gas phase to biosensor cells (AIRCHO-SEAP) genetically engineered to express the *Aspergillus nidulans*-derived transactivator AlcR that, in the presence of acetaldehyde, activates its cognate promoter PAIR, driving expression of the reporter gene SEAP (AIRCHO-SEAP cells). Ethylene is oxidized to acetaldehyde on PdCl2 with Cu+ based on the Wacker process. The generated acetaldehyde is captured by AIRCHO-SEAP and converted into SEAP expression, measured as a colorimetric signal .Indicators for the detection of these compounds, such as terpenes, carboxylic acids, alcohols, aldehydes, sulfur compounds, ammonia, and jasmonates, have been reported.

- A color-based pH indicator, developed using bromophenol blue immobilized on a cellulose membrane, enabled the detection of VOC (e.g., acetic acid) evolution in the headspace of guava packaging. This label provides the consumer with the freshness status of guava.

Biosensors for the detection of pathogens, allergens, and other toxicants, such as pesticides

- The need for simple, rapid, and field-portable analytical methods has boosted development of biosensors for food analysis.
- The integration of biomolecules, such as enzymes, immunosystems, tissues, organelles, or whole cells, with a variety of transduction methods, such as electrical, thermal, or optical signals, has enabled the development of a wide array of biosensing devices.
- Their selectivity and relative ease of analysis make them advantageous for use in food analysis.
- The development of biosensors in this field is described with biosensor examples for the detection of pathogens, allergens, and other toxicants, such as pesticides and mycotoxins.
- In this section, we briefly highlight the application of biosensors for allergens, toxicants, and pathogen detection.

Biosensors for Food-Allergen Detection

- The presence of allergens in food products such as milk, soybeans, crustaceans, eggs, gluten-containing cereals, peanuts, and nuts (e.g., almonds, Brazil nuts, cashews, walnuts) is an increased safety concern, as prevalence of food allergies due to even trace amounts of allergens is increasing.
- About 10% of preschool children in industrial countries suffer from clinical food allergies.
- A variety of DNA or immune-based biosensors have been developed for allergen detection, but in many cases sample preparation and purification are laborious and time-consuming.
- Employing antibody-based detection and magnetite beads, has commercially developed a sensor to detect peanut allergens in ppm.
- Peanut allergens were detected by surface plasmon resonance (SPR)-immune-based biosensor in chocolate candy bars.
- Colorimetric, silicon-based, optical thin-film biosensor chip with PCR amplification was developed with the ability to simultaneously identify eight food allergens found in soybeans, wheat, peanuts, cashews, shrimp, fish, beef, and chicken.

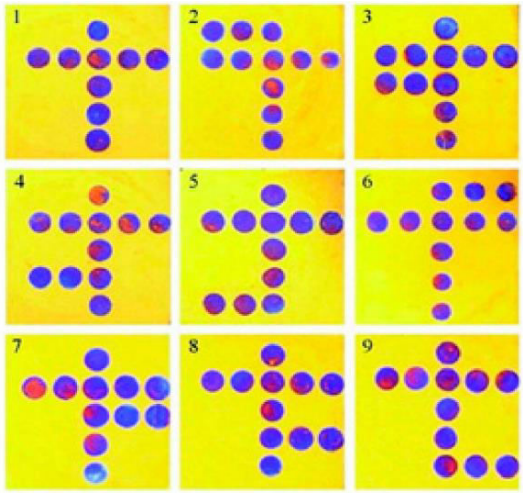


Figure 44. Allergen-detection chip, detection of corresponding allergens: 1, H_2O; 2, cashews; 3, peanuts; 4, wheat; 5, soybeans; 6, chicken; 7, fish; 8, shrimp; 9, beef

- The presence of gluten in food such as wheat, barley, and rye causes celiac disease for individuals who are unable to digest gluten.
- A label-free electrochemical immunological sensor for β-lactoglobulin, an allergen usually found in milk was developed.
- Allergy-causing proteins, such as casein and β-lactoglobulin (β-LG), in milk have also been detected based on aptamer recognition with the aptamer immobilized on a graphene-modified screen-printed electrode with voltammetric detection.

- A casein immunosensor with Localized Surface Plasmon Resonance (LSPR) detection and immobilized casein antibodies was reported to detect casein in raw milk with.
- Several types of electrochemical biosensors have been designed for lactose quantification utilizing coimmobilized β-galactosidase and glucose oxidase enzymes.
- Several enzymatic lactose sensors are also available for the detection of trace amounts of lactose in lactose-free milk products.

Biosensors for Bacterial-Pathogen Detection

- The main causes of these million foodborne illnesses diseases are pathogenic bacteria *Escherichia coli* and *Salmonella* spp.
- Rapid detection of pathogenic bacteria plays an important role in food analysis.
- The main methods for pathogen detection are based on Polymerase Chain Reaction (PCR) or plate counting, which require sample enrichment and long analysis time.
- Most biosensors for bacterial-pathogen detection are those based on immune and DNA recognition, but these require extensive preparation procedures, involve labeling, multiple washing steps, and specialized facilities.
- Alternatively, synthetic antimicrobial peptides have been proposed as recognition agents, enabling detection and quantification of four bacterial strains, *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*, and *Staphylococcus epidermidis*.
- Colorimetric biosensor strips fabricated with peptides immobilized on a gold chip were also reported for the detection of *Listeria monocytogenes* in milk and meat samples.
- Fluorescent DNAzyme probe that specifically binds *E. coli* was developed and printed on a cyclo-olefin polymer transparentv package.

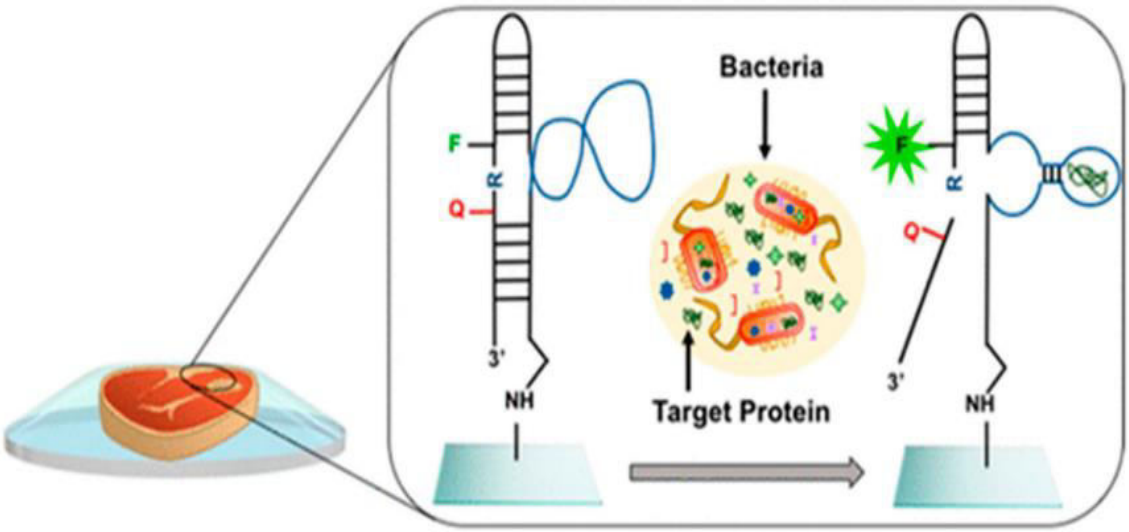


Figure 45. Fluorescent DNAzyme probe with specific binding characteristics for *E. coli.*

Biosensors for pesticide residues:

- The widespread use of pesticides in agriculture leads to their accumulation in soil, ground water, and crops.
- Due to their inherent toxicity, a control of pesticide levels in food products is necessary.
- Biosensors based on acetylcholinesterase (AChE) inhibition have been reported for the detection of carbamates and organophosphate used in insecticides.
- AChE immobilization approaches on screen-printed electrodes by was tested on bioincapsulation in sol-gel composites, metal-chelate affinity, and entrapment in a photopolymerizable polymer.
- Biosensors based on organ phosphorus hydrolase, which catalyzes the hydrolysis of organophosphorous pesticides, were also developed.
- Insecticides such as Organophosphates, Carbamates, Neonicotinoids, Pyrethroids, Organochlorines were tested by various optical sensors.

Figure 46. Basic principle of OPs biosensor by AChE enzyme

4.2 Monitoring of raw material conversions.

- To take up the challenge of world hunger, increased demand for food and biodiversity, new interdisciplinary strategies should be implemented to boost the current food production systems.
- From a technological point of view, such strategies should imply the development of more efficient and sustainable food production processes, with low environmental impact and ensuring high food quality standards.

- Since ancient times, microorganisms and active biomolecules have been employed for food and food additives production.
- Nowadays, food industry comprises a large variety of bioprocess technologies such as fermentations, biotransformations and downstream processes.
- These biotechnological processes are carried out in a well-controlled environment, with a high degree of automatization, and under continuous monitoring.
- Physical and chemical sensors are the most commonly used for food bioprocess monitoring to monitor raw material conversion.
- However, the detection of complex chemical compounds, relevant biomolecules or cells requires the use of more selective and specific analytical methods.
- In this context, biosensors constitute the most promised sensing technology for food bioprocess control and monitoring to optimize the conversion of raw material into final food product.
- Electrochemical biosensors are self-contained, integrated analytical devices, in which a biological recognition element is in intimate contact or incorporated with an electrochemical transducer, allowing the use of an electroanalytical technique (potentiometric, amperometric, conductometric, impedimetric, field effect, etc.) to measure the analytical response.
- These devices can detect target analytes by using catalytic (enzymes, cell, tissues, etc.) or affinity (antibodies, aptamer, lectins, DNA, etc.) bioreceptors.
- Electrochemical biosensors technology allows the design of miniaturized, portable and cost-effective sensors that exhibit the inherent specificity provided by the bioreceptor, combined with the high sensitivity and low detection limit of the physicochemical transducer.
- These biosensors can also provide rapid, on-site measurements with minimal or without sample preparation. Because of these relevant properties, electrochemical biosensors are promising alternatives to traditional methods for food safety and food processing analysis.

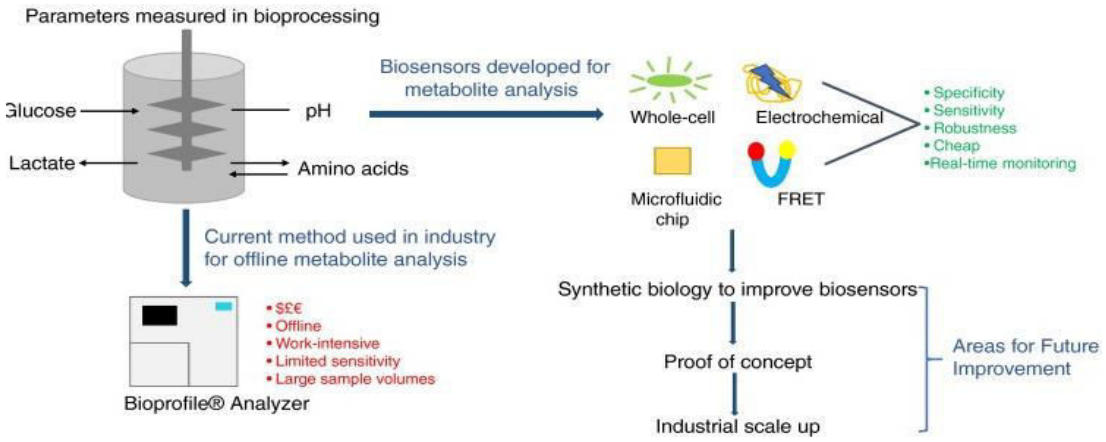


Figure 47. Bioprocess monitoring through biosensors

- In the last years, electrochemical biosensors have been mainly based on nanomaterials-modified electrodes due to the relevant properties of these materials for transduction.
- Nanomaterials have high surface area-to-volume ratio, allowing to immobilize bioreceptors in large yield with the consequent increase in the analytical sensitivity.
- These materials can be also chemically modified with a variety of ligands to allow easy immobilization of bioreceptors through covalent and non-covalent linkages.
- In addition, several nanomaterials have excellent electroconductive and electrocatalytic properties, allowing to construct biosensor devices with improved analytical characteristics.
- Enzymes are, by far, the biological receptors more commonly employed to construct electrochemical biosensors for food analysis.
- These biosensors have been mainly designed for food safety and food composition analysis as explained in the module 1.
- However, much of them can be easily adapted to monitoring food bioprocesses, such as fermentation, in which microorganisms are employed to produce and modify foods.
- These bioprocesses require the accurate control and monitoring of the concentrations of substrates and products,
- Electrochemical biosensors based on affinity recognition mechanisms have been proposed as alternatives to detect microbiological contamination and spoilage in food, as well as the presence of microbial toxins.
- Although these biosensors have been mainly conceived for food quality control, they can be also potentially employed for food bioprocess monitoring to keep check on raw material yield.
- Electrochemical biosensors are powerful alternatives to conventional laboratory techniques for food analysis, with potential application in food bioprocess monitoring.

4.3 Detection of crop diseases, pathogens in plants,

- When plants are exposed to pathogens they initiate protection reactions whose molecular mechanisms are very complex.
- At the early stages, when visual symptoms such as injuries on the leaf surface are absent, plants respond to the presence of a pathogen with physiological component such as the decrease of the photosynthesis rate, which induces an increase of fluorescence and heat emission.
- The possibility to distinguish distinctive contaminations in a similar plant is attractive, since plants can be influenced at the same time by numerous pathogens, for example, nematodes, fungi, bacteria, phytoplasmas, viruses and viroids that conventional strategies identify at a late symptomatic stage.

- Detection of plant infection utilizing electrochemical techniques has pulled in much intrigue in light of their basic instrumentation, high specificity, affectability, quick, and is economical with potential for applications in sub-atomic sensing instrument.
- Recently, nanomaterial-based electrochemical sensors have been reported for plant disease detection.
- The utilization of gold nanoparticle (AuNP) modified cathode for the electrochemical detection of methyl salicylate, a key plant volatile organic compound released by plants during infections.
- In case of DNA based electrochemical strategies, voltammetric examinations have been utilized as a basic device for a discriminative investigation of nucleic acid conformation and modification with the synchronous identification of all bases of the DNA without the need of a hydrolysis step.

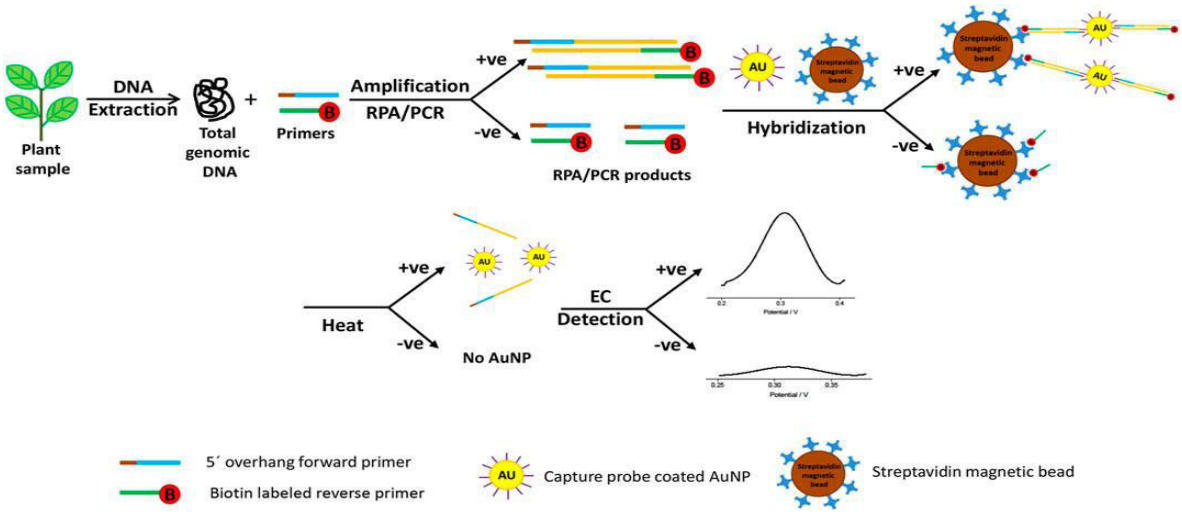


Figure 48. Schematic exemplum of the DNA-based electrochemical bioassay for plant pathogen detection

- The detection generally includes the redox labels physically or covalently linked to specific target DNA allocated on the active electrode surface.
- Current advance in DNA based biosensors for improvement of parallel microarrays and high-throughput outlines can be connected to DNA sequencing innovations.
- Although much research has been done on electrochemical biosensors for other areas like food quality but its practical application for plant disease detection is in pipeline for detailed investigation.
- Colorimetric biosensors are an appealing optical biosensor since one can undoubtedly and immediately see with the bare eye the presence of pathogenic microorganisms in the specimen

through a colour change without the requirement for any expository instrument or chemical reagent.

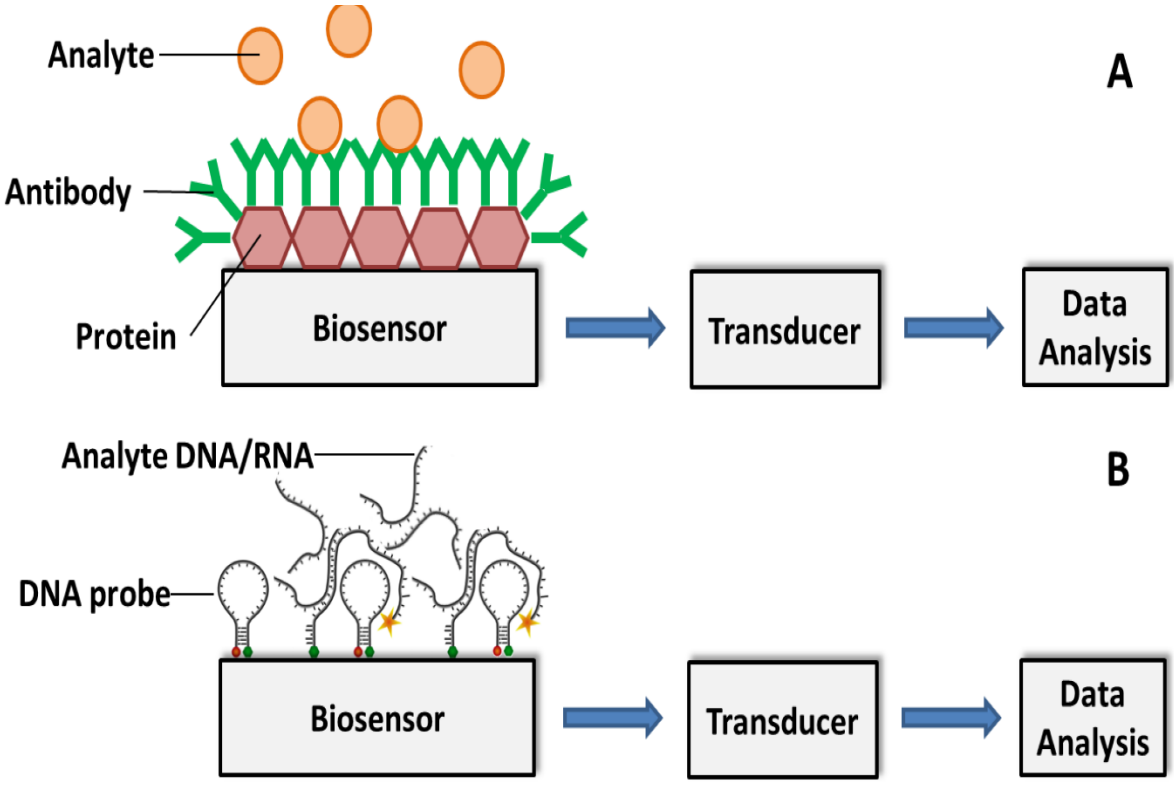


Figure 49. Biosensor concepts for plat disease detection

- Optical biosensors measure light assimilated or radiated as the after effect of a biological or synthetic response, while electrochemical biosensors depend on biochemical responses that cause electron exchange between a functionalized electrode and an analyte in solution, and can make amperometric, voltammetric, or impedimetric estimations.
- Another approach, nanotechnology has real way against numerous agricultural problems including plant disease identification as well as control.
- Nanoparticles show interesting electronic and optical properties and can be incorporated utilizing various kinds of materials for electronics and detecting applications.
- The prevalence of nanomaterials for sensor improvement could be credited to the friendly platform it facilitate the gathering of bio-recognition component, the high surface region, high electronic conductivity and plasmonic properties of nanomaterials that upgrade the constraint of detection.
- Now a day's nano-based materials is introduced which enhance the effectiveness of fungicides and pesticides, enabling minor dosages to be utilized.
- Additionally, nanodiagnostic and microfluidics offer novel tools to improve the sample preparation step that remains difficult to incorporate in a miniaturized platform.
- The signal intensification methodologies may perhaps challenge those of target enhancement.

- Fast nearby recognition of plant pathogens utilizing nanosensor, nanobased kits, nanobarcodes, nanobiosensors and other portable diagnostic systems can help agricultural and food industry to manage different plant diseases.
- The Cucumber Mosaic Virus (CMV) and Papaya Ring Spot Virus (PRSV) is a highly aggressive disease that can reduce yield and quality of the vegetable and fruit.
- Enzyme-based biosensors are based on using enzymes that are specific to the biomolecules under recognition to catalyze the generation of a product that can be evaluated by a transducer.
- A large portion of the enzyme utilized in biosensors is oxidases that respond with dissolved oxygen to produce hydrogen peroxide.
- Antibody-based biosensors with a range of transducing methods have been developed.
- Detection of phytopathogenic organism by immunological techniques depends on availability and affinity of selective antibody binding to the target bio-molecule.
- In case of complex plant material, sample is prepared by separation and accumulation of target molecules to facilitate effective pathogen sensing.
- Antibody-based sensors, also known as immunosensors involve the use of both polyclonal and monoclonal antibodies.
- Antibodies can be directly immobilized on the exterior of the transducer or attached to the surface of magnetic beads to perform immunomagnetic separation and detection.
- Utilizing biosensors and other compact demonstrative frameworks can definitely help the agriculture business.
- However several drawbacks of biosensor technology for on-site diagnosis of multiple pathogens includes sample preparation, limited life span of biological entity, weak selectivity in complex sample matrices, complexity of manipulations and obviously the high cost.

4.4 Detection of soil nutrients, pesticide and its residual detection.

- The soil N (nitrogen), P (phosphorus) and K (potassium) sensor is suitable for detecting the content of nitrogen, phosphorus, and potassium in the soil, and judging the fertility of the soil thereby facilitating the systematic evaluation of the soil condition.
- Can be buried in the soil for a long time, resistant to long-term electrolysis, corrosion resistance, vacuum potting, and completely waterproof.
- Soil NPK sensors are widely used in soil nitrogen, phosphorus and potassium detection, precision agriculture, forestry, soil research, geological prospecting, plant cultivation and other fields.
- Commercially available sensors has following features.

- Simple to use, few operation steps, fast measurement, no reagents, unlimited detection times.
- High measurement accuracy, fast response speed, and good interchangeability.
- The electrode is made of specially treated alloy material, which can withstand strong external impact and is not easy to damage.
- Completely sealed, resistant to acid and alkali corrosion, and can be buried in soil for long-term dynamic testing.
- The probe plug-in design ensures accurate measurement and reliable performance.
- Measurement of NPK contents of soil is necessary to decide how much extra contents of these nutrients are to be added in the soil to increase crop fertility.
- This improves the quality of the soil which in turn yields a good quality crop.
- The color sensor is based on the principle of absorption of color by solution.
- It helps in determining the N, P, K amounts as high, medium, low, or none.
- The sensor probe along with proper signal conditioning circuits is built to detect the deficient component of the soil.
- It is useful in dispensing only required amount of fertilizers in the soil

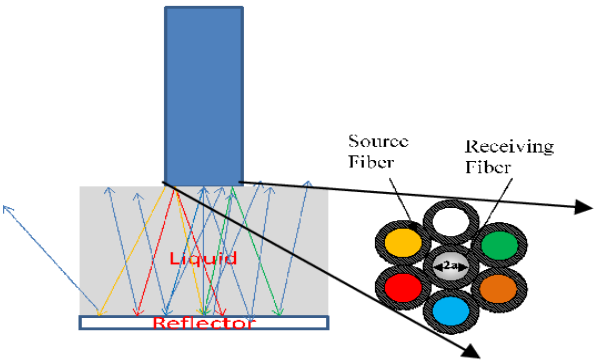


Fig 50 Working principle of soil NPK fiber optic sensor

- Plenty of electrochemical sensors, such as potentiometric ion-selective electrodes (ISEs), and ion-selective field-effect transistors (ISFETS); impedimetric sensors with solvent polymeric membranes; and optical sensors, have been developed for nitrogen's ionic forms in soils.
- The solubility of phosphates depends on the pH of the soil: below pH 7, $H_2PO_4^-$ is prevalent, and therefore, the majority of potentiometric ISEs have been developed for detection of this ion in soils, even if the development of highly selective potentiometric sensors for detection of the hydrophilic phosphates is a rather challenging task.
- Novel ionophores and sensing ligands, permitting selective binding of $H_2PO_4^-$ and HPO_4^{2-} ions, should be employed inside sensing membranes in order to replace the classical anion-exchangers having the lowest selectivity to these hydrophilic ions according to the Hoffmeister selectivity sequence determined by the free energy of ion solvation.

- Electrochemical and optical sensors have been developed previously for potassium assessments in soils.
- Among the soil moisture sensors, capacitive sensors are the most exploited.
- Electrochemical microfluidic paper-based analytical device (μPAD) used to monitor the inhibition of the respiratory activity of Escherichia coli following exposure to heavy metals, pesticides, and penicillin sodium.

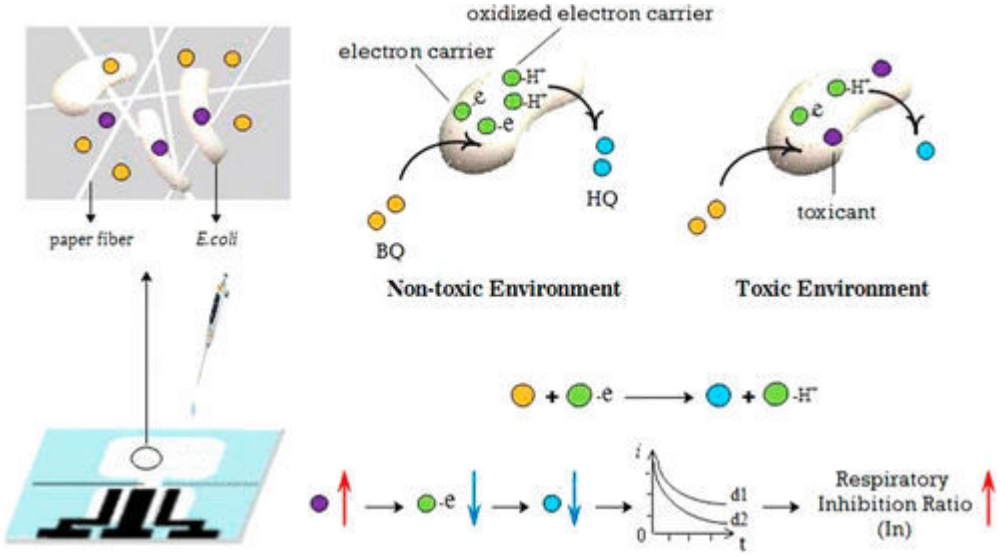


Figure 51. Electrochemical μPAD used to monitor the inhibition of the respiratory activity of E. coli following exposure to heavy metals, pesticides, and penicillin sodium.

- Electrochemical biosensors reported for pesticide sensing applications include those based on (1) enzymes, (2) whole cells, and (3) antibody–antigen interaction (immunosensors)
- A direct mode enzymatic biosensor measures analyte concentration or product formation during enzymatic reactions, whereas indirect mode biosensors monitor enzyme inhibition due to contact with the analyte of interest.
- Acetylcholine esterase (AChE) is an enzyme mainly found in cholinergic neurons and catalyzes the hydrolysis of the neurotransmitter acetylcholine to choline and acetate, as di Organophosphates inhibit AChE activity through phosphorylation of the serine residue of the enzyme's active center, preventing the hydrolysis of acetylcholine discussed earlier.
- The biosensor designed by (1) cross-linking AChE with glutaraldehyde, (2) immobilization of the cross-linked enzyme on the surface of a glassy carbon electrode modified with semiconducting single-walled carbon nanotubes (s-SWCNTs), and (3) treatment with bovine serum albumin (BSA) to prevent nonspecific binding.
- In addition to AChE, other enzymes such as butyrylcholinesterase (BChe), choline oxidase (ChOx), phosphotriesterase (PTE), and organophosphorus hydrolase (OPH) have been successfully utilized for electrochemical biosensing of organophosphate pesticides.

- The biosensor had the ability to detect different classes of pesticides, including carbendazim (benzimidazole), chlorpyrifos (organophosphate), DDT (organochlorine), dinocap (dinitro-octylphenyl crotonate), and ethion (organophosphate).
- Microbial biosensors have been reported for the detection of several classes of pesticides, including organochlorines, triazines, and organophosphates.
- Microbial biosensors developed based on the inhibition of the photocurrent generated by the cyanobacterial species *Anabaena variabilis*.
- In addition to microbial cells, mammalian cells have been successfully utilized in biosensing applications.

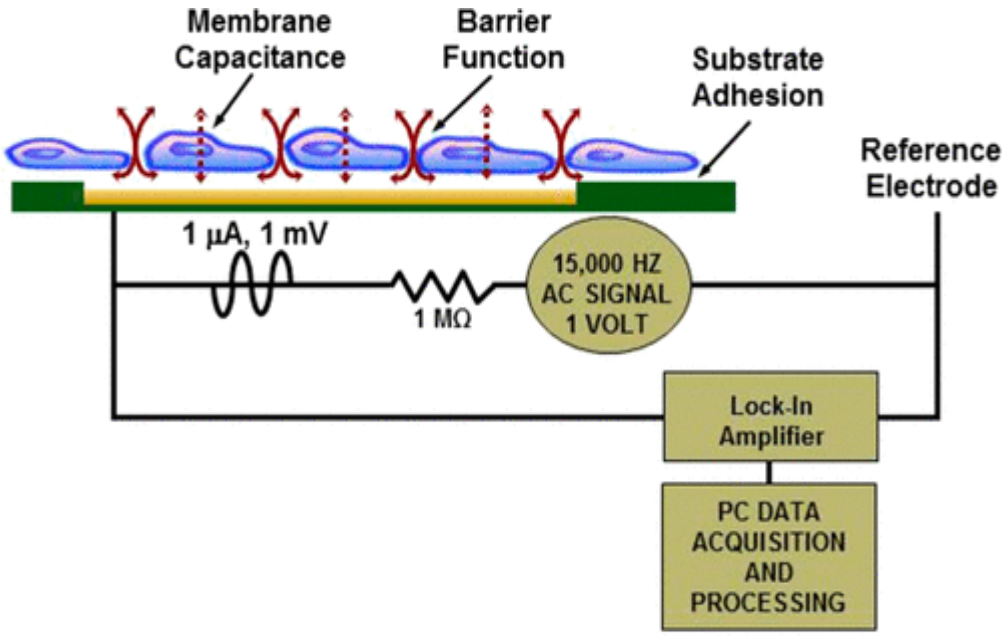


Figure 52. Cells adhered to the electrode surface are exposed to a high-frequency, low-voltage, and amperage ac signal with the monolayer of cells impeding the flow of electrons. Upon exposure to toxicants, the cells detach from the electrode surface, resulting in a decrease in measured impedance

Module-5

Applications of Nanomaterials in Biosensors

5.1 Nano Materials in biosensors; Carbon based Nano Material, Metal oxide and nano particle, Quantum dots,

- The nanomaterials have aroused much interest soon after the discovery of nanostructures in the early meteorites. Synthesis of gold NPs was the first
- Nanomaterials can be defined as a set of materials having at least one dimension less than w100 nanometer (nm or 10^9 m) or 1-00 nm.
- 1 nm is one-millionth of a millimeter or w 1, 00,000 times smaller as compared to the diameter of a human hair. Some nanomaterials can be found naturally.
- Nanomaterials can be designed with specific applications and are being used already for various commercial products and processes.
- Because of their small size, nanomaterials exhibit unique or novel properties.
- The length scale indicating the size of nanomaterials in comparison to various biological components is shown below.

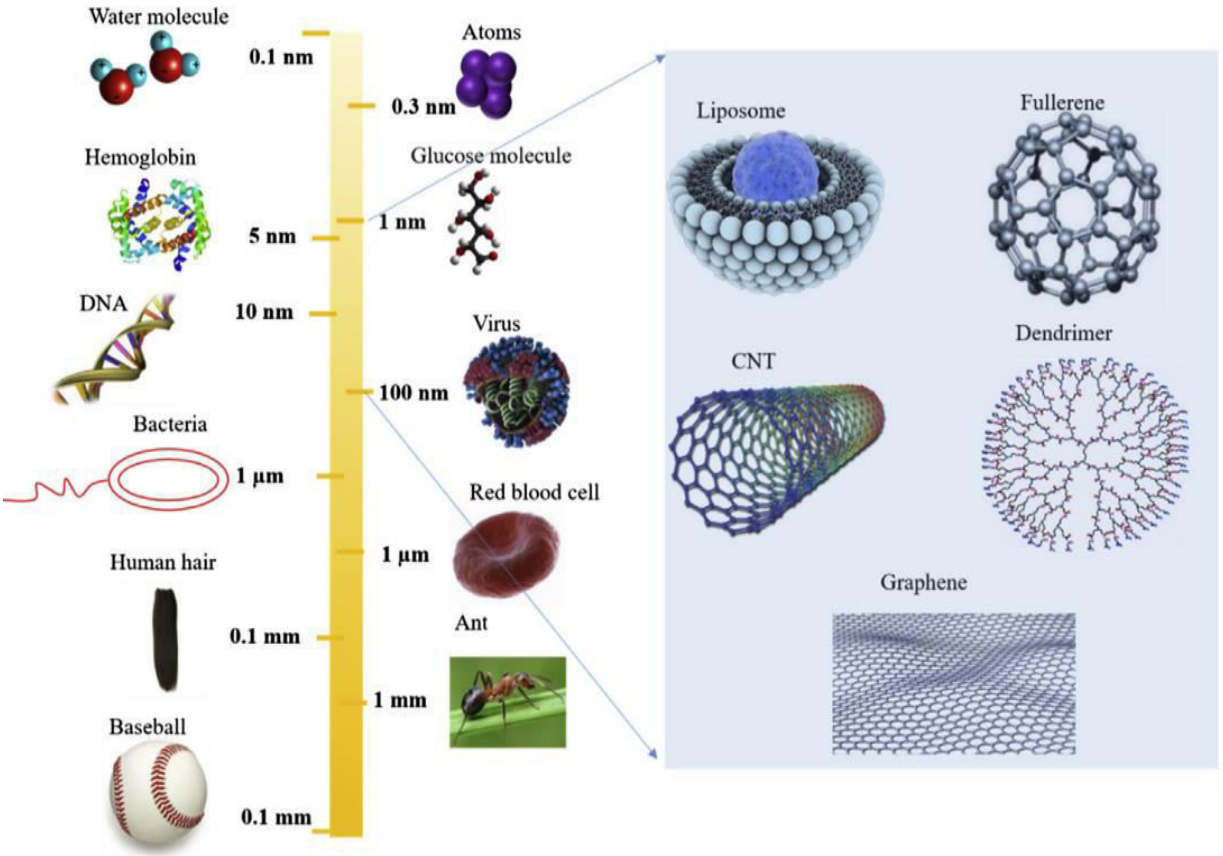


Figure 53. Length scale of nanomaterials

- Atoms are the basic building blocks of nature.
- The size of an atom is measured in angstrom: 10^{10} m or 0.1 nm which is comparable with the Bohr radius (0.5 nm).
- Two atoms provide a molecule, e.g., fullerene contains 100 atoms.
- The quantum effect is an important factor for the size of a semiconductor particle.
- The Bohr radius of a particle can be defined as $a_b = \varepsilon a_0 . m/m^*$, ε is a dielectric constant of a material, m is the rest mass of the electron, m* is the particle mass, and a_0 is the Bohr radius.
- A 1 mm-sized cube crystal of salt can contain 10^{19} atoms or more, thus a bulk material can be made by a number of atoms.
- Similarly, the building blocks of living organisms are cells (size 1 mm) that consist of thousands of small and large molecules that are much larger than a NP.

Properties of nanomaterial

- The materials at the nanoscale have interesting properties as described below:
- **Surface Area:** Suppose a cube having a size of 1 cm x 1 cm x 1 cm is cut into several equal pieces ending in cubes of size 0.1 mm x 0.1 mm x 0.1 mm each.
- If we cut the cube, the volume of all small cubes would be the same as that of the starting cube.
- The surfaces of all cubes have 100 times more area than that of the cube with which we started.
- And if we cut more cubes of the size of 1 nm x 1 nm x 1 nm, the area of the surfaces will increase 10 million times as compared with that of the original cube.
- This is how nanomaterials have extremely high surface-to-volume ratio.
- This allows for nanomaterials to interact with the environment or other materials strongly compared with bulk materials.
- In a material, the interior atoms are much more coordinated due to more bonds than surface atoms, resulting in stable atoms.
- At corners and edges, the atoms have less coordination leading to lesser stability than the interior atoms.
- The surface of a nanomaterial becomes quite reactive with nanoscale dimensions material and shows extraordinary catalytic and absorbance activity.
- **Quantum Confinement**: In quantum mechanics, the characteristic radius of an electron is defined as Bohr exciton radius.
- In bulk semiconductor materials, the radius of electron mobility is known to be higher than the Bohr exciton radius, with the result that mobility is not disturbed or not confined.
- The quantum confinement effect can be found when the size of the particle is too small to be comparable with the wavelength of the electron.

- When a particle size of a material becomes too small or comparable with Bohr exciton radius the electron mobility is confined.
- The electrons and holes are thus squeezed into small particles resulting in "quantum confinement" of the electron-hole pairs.
- The confinement means restricting the motion of randomly moving electrons to specific energy levels (discreteness).
- If a particle is of nanoscale dimensions the confining dimensions make energy levels discrete and this will increase or widen the material band gap or energy gap.
- When the size of a particle becomes nearly Bohr exciton radius, the excitonic transition energy, blue shift in the absorption, and luminescence band gap energy increases due to the quantum confinement effect.

Classification of nanomaterials

- Nanomaterials can be classified as per the size and dimensions.
- There are four types of nanomaterials such as zero dimension, one dimension, two dimensions, and three dimensions.
- **Zero-dimensional:** In zero-dimensional (0D) nanomaterials, all three dimensions of materials exist in nanoscale, e.g, NPs such as gold,palladium, platinum, silver, or quantum dots.
- NPs can be spherical in size with a diameter of 1e50 nm. It has been found that some cube and polygon shapes constitute 0D nanomaterials.
- **One-dimensional:** These nanomaterials having one dimension are in the range of 1e100 nm and the other two dimensions can be in macroscale.
- Nanowires, nanofibers, nanorods, and nanotubes are examples of one-dimensional (1D) nanomaterials.
- Some metals (Au, Ag, Si, etc.), metal oxides (ZnO, TiO2, CeO2, etc.), quantum dots, and others can provide 1D nanostructures.
- **Two-dimensional**: In this class of nanomaterials, two dimensions are in nanoscale and one dimension is in macroscale. Nano thin-films, thin-film multilayers, nanosheets, or nanowalls are two-dimensional (2D) nanomaterials.
- The area of 2D nanomaterials can be several square micrometers keeping thickness always in the nanoscale range. 4. Three-dimensional: In three-dimensional (3D) nanomaterials, there are no dimensions in nanoscale, and all dimensions are in macroscale. Bulk materials are 3D nanomaterials that are composed of individual blocks which may be in nanometer scale (1e100 nm) or more.

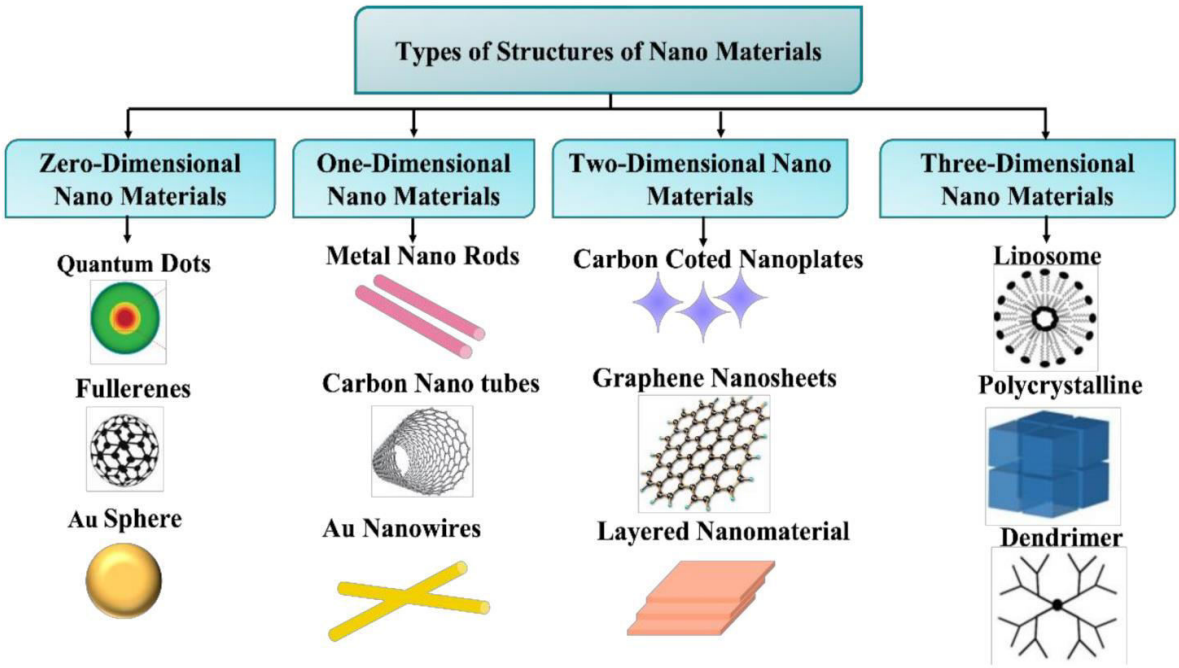


Figure 54. Different types of nanomaterial

5.1.1 Carbon based nanomaterials

- In recent years, carbon-based nanomaterials derived from a range of forms, from C60, to CNTs to graphene have become the most active research field in nanoscience, effectively promoting the rapid development of nanotechnology.
- Carbon-based nanomaterials usually have good electrical conductivity and biocompatibility, and can improve the active sites of electrochemical reactions.
- Due to the large specific surface area that increases the amount of immobilized biomolecule, this kind of materials are of interest in the field of electrochemical research.

Carbon Nanotubes (CNTs)

- Carbon nanotubes (CNTs) are considered as a derivative of both carbon fibers and fullerene with molecules composed of 60 atoms of carbons arranged in particular muffled tubes.
- Carbon nanotubes (CNTs) are considered as a derivative of both carbon fibers and fullerene with molecules composed of 60 atoms of carbons arranged in particular muffled tubes Among one-dimensional nanomaterials CNTs have both a special structure (radius: 2–20 nm, with axial dimensions on the micron scale) and a large specific surface area.
- The carbon atoms in CNTs form a wide range of delocalized bonds with significant conjugation effects, resulting in substantive conductivity.
- There are four different categories of CNTs, Categories of carbon nanotubes: (a) armchair, (b) zigzag, and (c) chiral.

- Based on these excellent features, CNTs have a very wide range of applications in immunosensors.
- Among these, the use of CNTs as electrode modifying substrates or markers to build highly sensitive sandwich-type immunosensors represent an important aspect of their study.

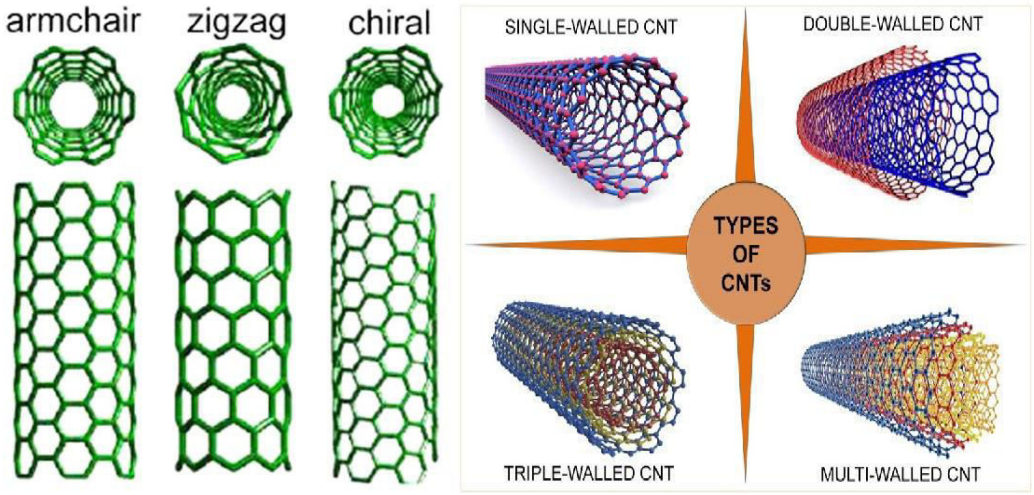


Figure 55. Different types of CNTs

- CNTs have good intermolecular electron transfer properties, when they are linked to horseradish peroxidase (HRP) to modify the electrode.
- Sandwich electrochemical immunosensor developed using multi-walled CNTs includes the following steps: (1) CNTs with specific surface area were used to immobilize the primary antibody on the electrode surface; (2) a sandwich reaction was performed to capture enzyme-labeled secondary antibodies loaded on CNTs on the electrode surface; (3) the addition of H_2O_2 to the substrate to reflect the concentration of the antigen.

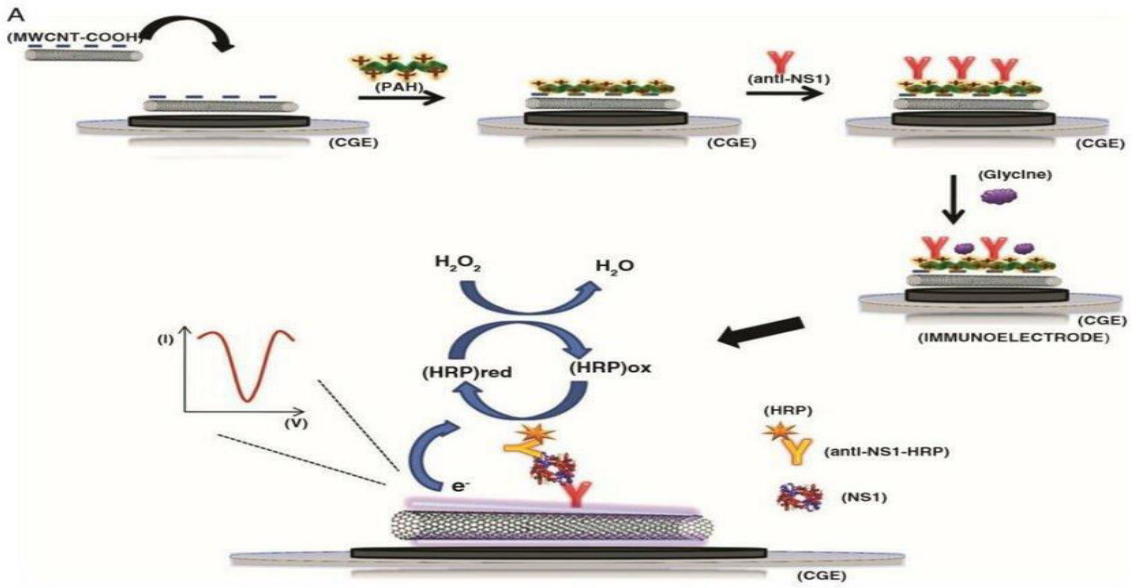


Figure 56 Sandwich electrochemical immunosensor based on CNT

- Graphene and Graphene Oxide Graphene exhibits unique physical and chemical properties, in particular a monolithic structure, high conductivity, large specific surface area, no toxicity and good electron mobility, and is widely used in the fields of electrochemical sensing and biosensing.
- The graphene materials carries a high density of defects on the surface and demonstrates particularly impressive positive electrochemical properties.
- To date, graphene-modified electrodes have been successfully applied for assessing H_2O_2, NADH, dopamine, ascorbic acid, uric acid and acetaminophen.

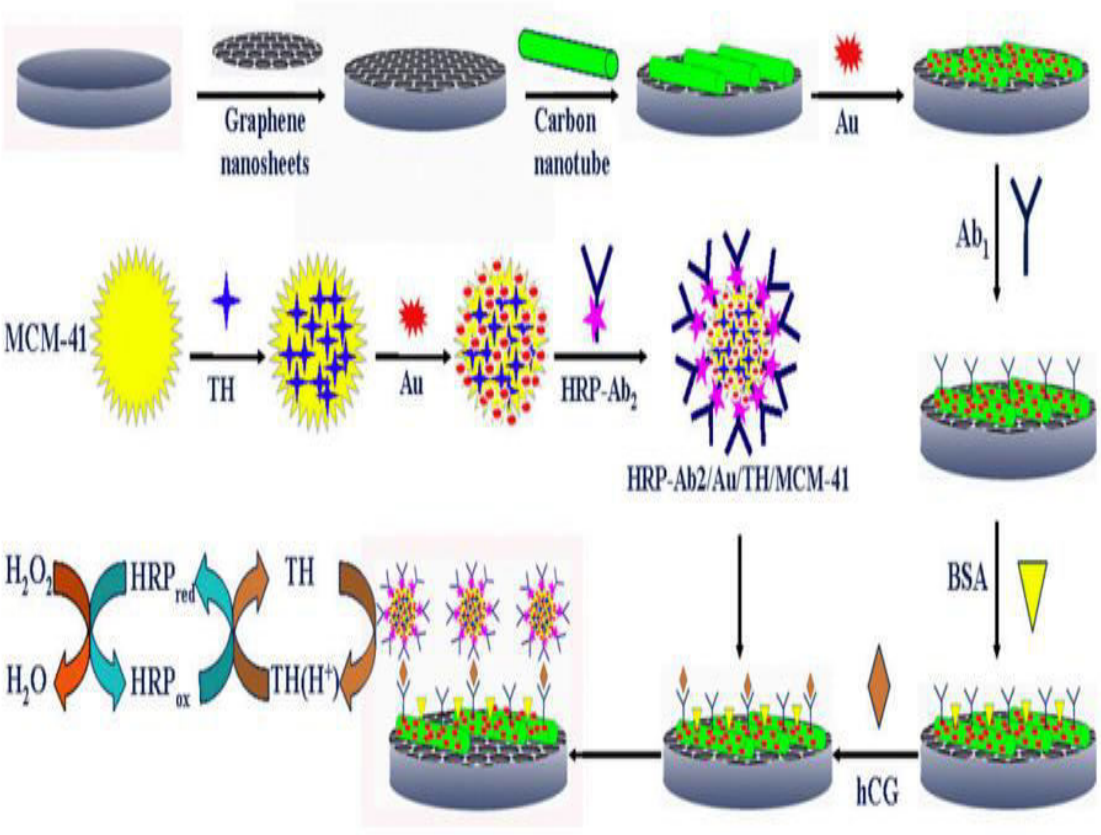


Figure 57 Graphene base electrochemical sensors

- The application of graphene onto a variety of inorganic and organic electroactive materials is lead its role as a promising new carbon substrate in the field of electrochemical analysis.
- Carbon nanofibers have a greater functional surface area and more surface active groups, and thus are considered to be a more promising material.
- Carbon nanospheres have relatively richer functional groups with better biocompatibility, dispersibility, and relative activity.

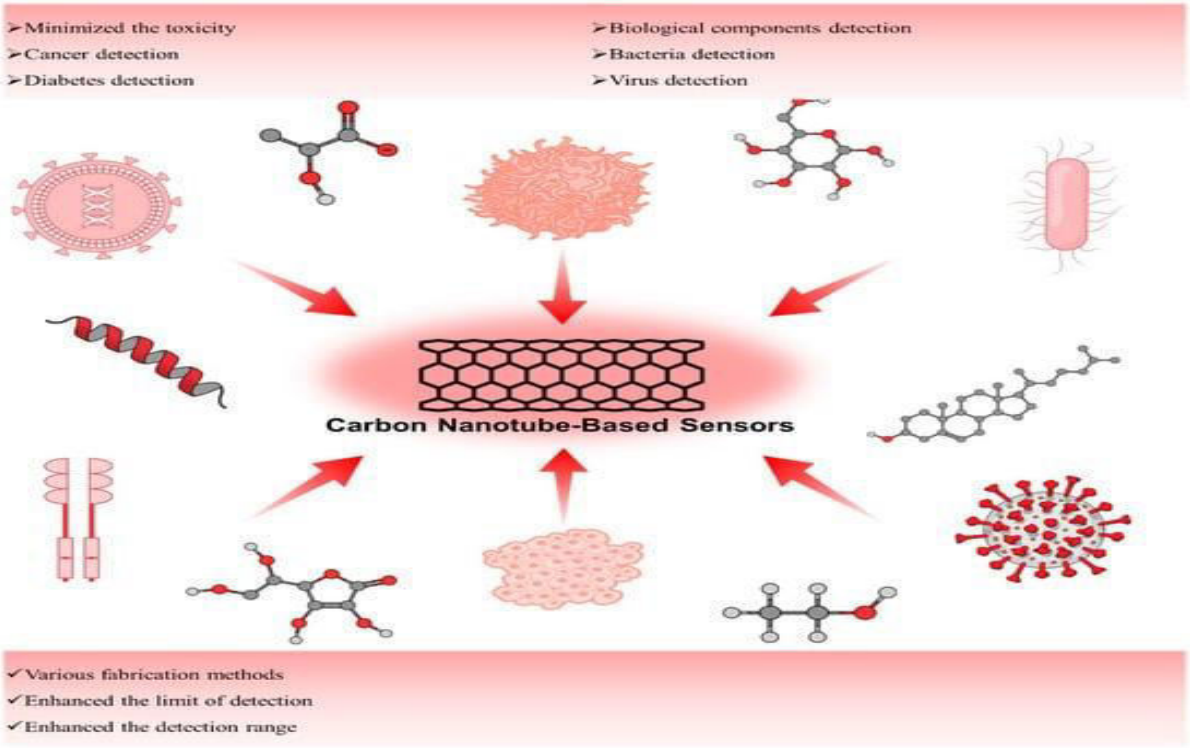


Figure 58 Schematic presentation of CNT-based biosensors

Metal oxide nano particle

- Metal oxide nanoparticles (MONPs) are of particular interests and have received much attention because of their unique physical, chemical and catalytic properties.
- We take an example of enzymatic biosensors based on (a) zinc oxide nanoparticle-based enzymatic biosensors, (b) titanium oxide nanoparticle-based enzymatic biosensors; (c) iron oxide nanoparticle-based enzymatic biosensors, and (d) on other metal oxide nanoparticle-based enzymatic biosensors.
- Substantial advances have been made in MONPs-based enzymatic biosensors.

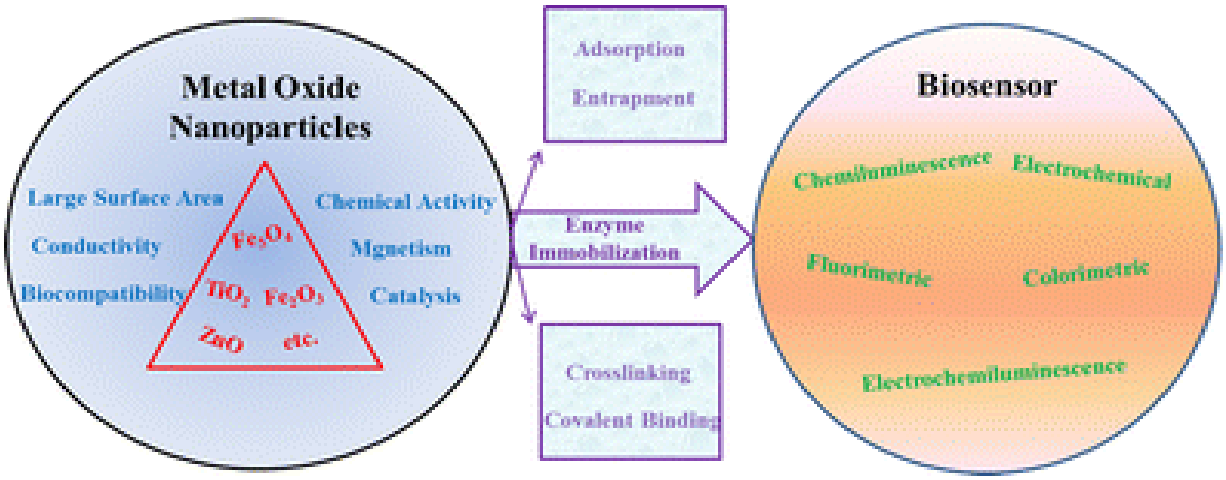


Figure 59. MONPs-based enzymatic biosensors

- Metal oxide nanoparticles (MONPs) have attracted much attention for their optical, magnetic and electronic properties.
- It is well known that MONPs not only have high surface area, good biocompatibility and chemical stability, but also display fast electron transfer ability.
- All these features make MONPs ideal immobilization matrices as well as transduction platform and/or mediators.
- Nanofabricated metal oxides also have been successfully applied in the field of biosensing system.
- It is well known that enzymes and enzyme mimetics can offer great amplification power through efficient catalytic turnover of substrates.
- The combination of enzymes with MONPs could provide a versatile platform for biosensing with various signal readout strategies, such as electrochemical, colorimetric, fluorimetric, chemiluminescence and so on.
- (a) **zinc oxide nanoparticle-based enzymatic biosensors** :As a biomimetic material, ZnO provides a versatile platform for biomolecules loading. ZnO nanoparticles with high isoelectric point are suitable for the adsorption of low-isoelectric point enzymes for biosensor fabrication.

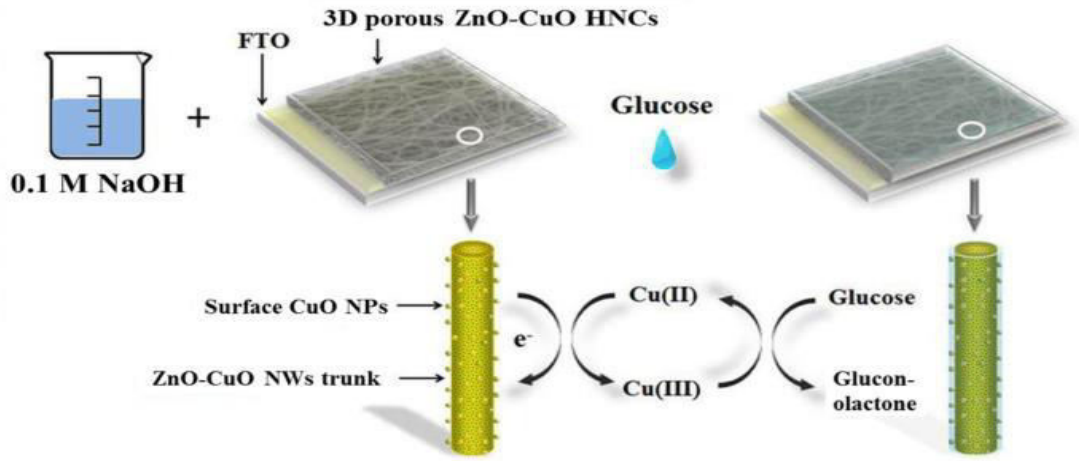


Figure 60. Schematic illustration of fabricated electrode and glucose detection mechanism over 3D porous ZnO–CuO HNCs surface

- (b) **Titanium oxide nanoparticle-based enzymatic biosensors**: TiO_2 is one of the most prominent materials in various applications related to catalysis, photovoltaic devices, sensors and paintings.
- c) **Iron oxide nanoparticle-based enzymatic biosensors**: Well-established magnetic nanoparticles with appropriate surface chemistry have been widely used in pollution remediation, chemical or biological separation, bioimaging, targeted drug delivery, diseases diagnostics and therapy, enzyme immobilization, biosensing, etc.

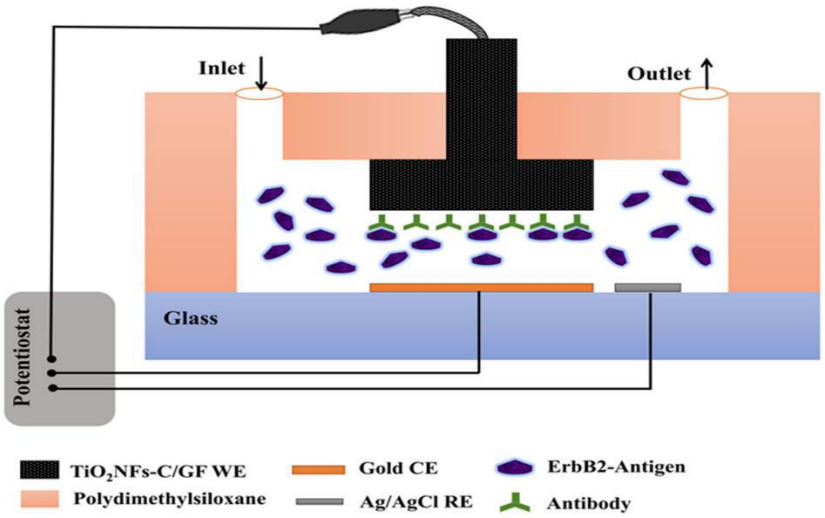


Figure 61. Schematic representation of the configuration of the microfluidic biosensor with 3D porous GF electrode modified with carbon-doped TiO2 NFs for the detection of breast cancer biomarkers

- d) **Other metal oxide nanoparticle-based enzymatic biosensors**: zirconia nanoparticles and their nanocomposites have been reported to be used for enzyme immobilization, such as HRP, GOx, AChE, Hb and Bilirubin oxidase. An electrochemical quartz crystal microbalance (EQCM) immunoassay was developed utilizing ZrO2 nanoparticles for selective capture phosphorylated AChE (Phospho-AChE) due to their strong affinity for phosphoric groups.

Quantum Dots

- Quantum dots are tiny specks of material, so small that some people say they have no dimensions. They exist as points of materials, typically 1/10,000 the size of a human hair.
- A variety of quantum dots not only have the characteristics of nanomaterials, but also can give good, sharp and sensitive dissolution peaks through electrochemical dissolution voltammetry.
- Quantum dots are nanoparticles made from semiconducting materials.
- The dots show quantum effects because they are so little.
- This means that electrons inside the dot are trapped and can only occupy defined energy levels.
- With only confined, discrete energy levels available, quantum dots can have different optical and electrical properties to a large quantity of the same material.
- This makes quantum dots useful in developing nanotechnology.
- Quantum dots can capture light and convert it into electricity.
- They can do this efficiently and require less space than larger, conventional materials.
- Changing the size of the quantum dot allows us to tune their ability to absorb and emit specific light frequencies.

- Within the chemical growth process, we can use flowing gases and cold temperatures to control the size of the quantum dot.
- A bigger dot will emit light at a longer wavelength. In the visible range, larger dots glow red. Smaller dots shine blue.
- Quantum dots confine the motion of electrons in all three spatial directions.
- This restriction leads us into the quantum world, and quantum dots' electrical and optical properties.
- The QD-based Fluorescence Resonance Energy Transfer (FRET) genosensor, most widely used in the biomolecule detection fields, and QD-based nanosensor for Rev-RRE interaction assay are presented as examples.
- Fluorescence resonance energy transfer (FRET)* is a distance-dependent physical process by which energy is transferred nonradiatively from an excited molecular fluorophore (the donor) to another fluorophore (the acceptor) by means of intermolecular long-range dipole–dipole coupling.
- FRET can be an accurate measurement of molecular proximity at angstrom distances (10–100 Å) and highly efficient if the donor and acceptor are positioned within the Förster radius (the distance at which half the excitation energy of the donor is transferred to the acceptor, typically 3–6 nm).
- The efficiency of FRET is dependent on the inverse sixth power of intermolecular separation, making it a sensitive technique for investigating a variety of biological phenomena that produce changes in molecular proximity
- In recent years, QD-based biosensors have emerged as a new class of sensor and are expected to open opportunities in plant virus detection, but as yet there have been very few practical applications.

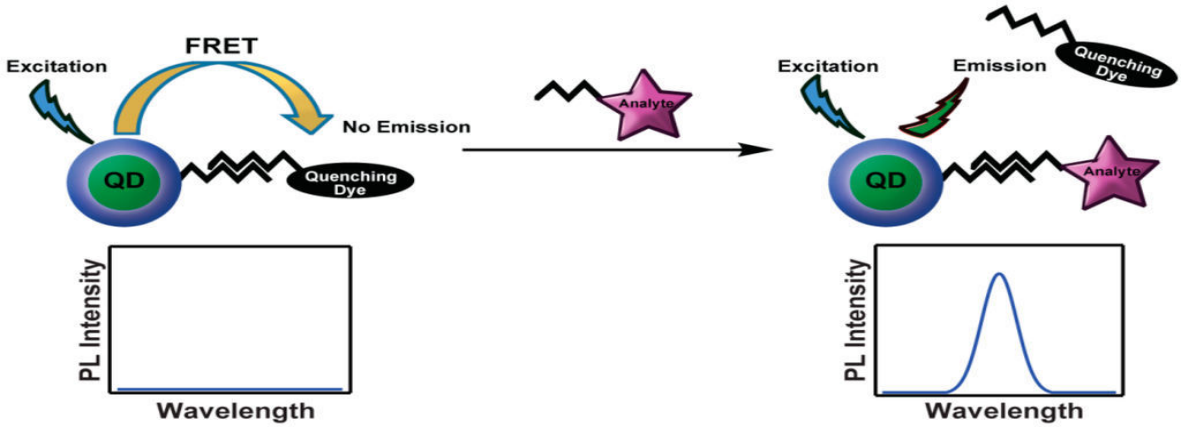


Figure 62. Competitive displacement of a quenching dye by the analyte removes the FRET interaction, resulting in QD emission recovery.

- Thus, quantum dot materials have good prospects in the related fields of nanomaterials and electrochemical analysis.
- Different semiconductor nanomaterials (ZnS, CdS, PbS and CuS) were used for labeling various proteins (α2-microglobulin, IgG, bovine serum albumin, C-reactive protein).
- According to the peak position of the marker and the tested peak current from the stripping voltammetry, different antigens were identified and determined.
- Sandwich-type immunosensor fabricated on an ITO chip covered with a well-ordered AuNPs monolayer applying CdTe quantum dots as electrochemical and fluorescent labels.

5.2 Role of nano material in Signal Amplifications, Detection and Transducer Fabrication

- Nanomaterials have recently aroused much interest due to the increased need for control of desired molecules present in the human body and environment.
- A nanomaterial comprises of nanoparticles (NPs) that are less than 100 nm at least in one dimension.
- The term "nanotechnology" deals with small-sized materials when the size is down to subnanometer or several hundred nanometers.
- The controlled synthesis and tuning properties of nanomaterials require knowledge of different disciplines such as physics, chemistry, electronics, computer science, biology, engineering, agriculture, etc. that may lead to the emergence of novel and multifunctional nanotechnologies.
- In this context, the exciting properties of nanomaterials have attracted the world scientific community toward their application in various sectors such as health, food, security, transport, and information technology, etc.

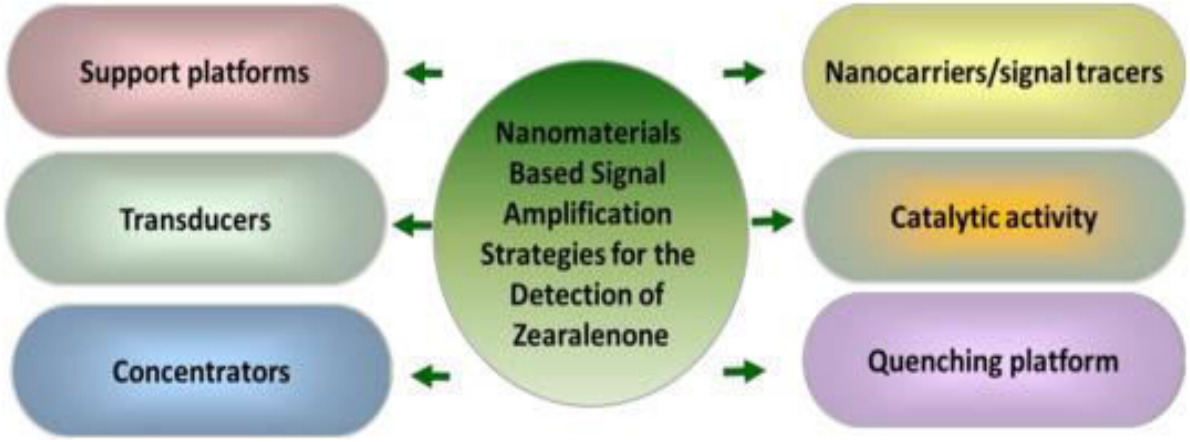


Fig. 63. The roles of nanomaterials in signal amplification.

- The intelligent use of nanomaterials is predicted to enhance the performance of biomolecular electronic devices with high sensitivities and detection limits.
- Diagnostics is clinically important both for identification of a disease and therapeutics.
- Early diagnostics plays an important role to detect a disease (prevention) or the outcome of a disease (prognosis).
- A number of biodevices have been fabricated for studies of blood gas, glucose/lactate/cholesterol, nucleic acid sequence analysis, proteins/peptides, combinatorial synthesis, toxicity monitoring, immunoassays, environment, defense, and forensic analysis.
- A biosensor for blood glucose monitoring has been successfully commercialized.
- Efforts are being made to enhance the resolution, accuracy, and miniaturization of biosensors for detection of biomolecules along with microfluidics and sample preprocessing.
- Because of their portability, the short time to obtain results, biosensors have been predicted to fulfill a number of unmet needs in the diagnostics industry.
- Microfluidic biosensing devices offer important opportunities for research, especially for clinical diagnosis, due to their numerous advantages.
- These miniaturized devices require minute volumes (10−9 to 10−18 L) in micron-sized channels and containers leading to the development of a "lab-on-a-chip".
- Nanomaterials are currently undergoing rapid development due to their potential applications in the field of nanoelectronics, catalysis, magnetic data storage, structural components, biomaterials, and biosensors.
- The use of NPs, nanotubes, and nanowires, etc. in biosensor diagnostic devices are being explored.
- With the advancement in properties of nanomaterials, their dimensions at the nanoscale level, new biodevices (smart biosensors) that can detect minute concentration of a desired analyte are emerging.
- Nanomaterials are generally used as transducer materials that are an important part for biosensor development.
- A biosensor consists of four parts namely (1) bioreceptor, (2) a transducer, (3) a signal processor for converting electronic signal to a desired signal, and (4) an interface to display.
- A variety of samples such as body fluids, food samples, and cells culture can be explored to analyze using biosensors.
- The engineered nanomaterials provide higher electrical conductivity, have nanoscale size, can be used to amplify desired signals, and are compatible with biological molecules.

- For example, carbon materials can be utilized for conjugation of biomolecules (enzyme, antibody, DNA, cell, etc.).
- It has been found that the use of nanomaterials may lead to increased biosensor performance including increased sensitivities and low limit-of-detection of several orders of magnitudes.
- Nanostructured materials show increased surface-to-volume ratio, chemical activity, mechanical strength, electrocatalytic properties, and enhanced diffusivity.
- Nanomaterials have been predicted to play an important role toward the high performance of a biosensor.
- To probe biomolecules such as bacteria, virus, DNA, etc. biocompatibility of nanomaterials is an important factor for designing a biosensor.
- Nanomaterials with various applications for biosensor development are discussed in this chapter.
- An important challenge is the standardization of immobilization procedure that can be utilized to intimately conjugate a biomolecule onto a nanomaterial.
- Therefore, the technique used to immobilize a given enzyme is one of the key factors in developing a reliable biosensor.
- A nanomatrix can be an excellent candidate to immobilize biomolecules on a transducer surface that can efficiently maintain bioactivity of the biomolecules.
- There are still many challenges such as miniaturization, automation, and integration of the nanostructured-based biosensors.

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ANALYSIS OF DAIRY PRODUCTS LAB		Semester	III
Course Code	BBTL358C	CIE Marks	50
Teaching Hours/Week (L:T:P: S)	0:0:2:0	SEE Marks	50
Total Hours	15	Total Marks	100
Credits	01	Exam Hours	2
Examination type (SEE)	PRACTICAL		

Course objectives:

- To learn preparation of sample of various dairy products for analysis
- To lean the detection of ingredients and adulterants in milk and milk products

Sl.No	Experiments
1	Preparation of sample for milk
2	Detection of adulterants in milk
3	Detection and quantification of starch in milk
4	Detection of cellulose in milk
5	Detection of added urea in milk
6	Detection of foreign fat in milk
7	Detection of gelatine in milk
8	Determination of pH in Whey powder.
	Demonstration Experiments
9	Preparation sample of curd and determination of total solids, moisture and fats
10	Preparation sample of curd condensed/flavoured milk and determination of titrable acidity.
11	Preparation sample of dried milk and determination of carbohydrates, protein and ash
12	Preparation sample of butter and determination of free fatty acids and moisture

Course outcomes (Course Skill Set):

At the end of the course the student will be able to:

1. Prepare various milk and milk products for analysis.
2. Learn to detect ingredients present and adulterants added to milk and milk product.

Assessment Details (both CIE and SEE)

The weightage of Continuous Internal Evaluation (CIE) is 50% and for Semester End Exam (SEE) is 50%. The minimum passing mark for the CIE is 40% of the maximum marks (20 marks out of 50) and for the SEE minimum passing mark is 35% of the maximum marks (18 out of 50 marks). A student shall be deemed to have satisfied the academic requirements and earned the credits allotted to each subject/ course if the student secures a minimum of 40% (40 marks out of 100) in the sum total of the CIE (Continuous Internal Evaluation) and SEE (Semester End Examination) taken together.

Continuous Internal Evaluation (CIE):

CIE marks for the practical course are **50 Marks**.

The split-up of CIE marks for record/ journal and test are in the ratio **60:40**.

- Each experiment is to be evaluated for conduction with an observation sheet and record write-up. Rubrics for the evaluation of the journal/write-up for hardware/software experiments are designed by the faculty who is handling the laboratory session and are made known to students at the beginning of the practical session.
- Record should contain all the specified experiments in the syllabus and each experiment write-up will be evaluated for 10 marks.
- Total marks scored by the students are scaled down to **30 marks** (60% of maximum marks).
- Weightage to be given for neatness and submission of record/write-up on time.

- Department shall conduct a test of 100 marks after the completion of all the experiments listed in the syllabus.
- In a test, test write-up, conduction of experiment, acceptable result, and procedural knowledge will carry a weightage of 60% and the rest 40% for viva-voce.
- The suitable rubrics can be designed to evaluate each student's performance and learning ability.
- The marks scored shall be scaled down to **20 marks** (40% of the maximum marks).

The Sum of scaled-down marks scored in the report write-up/journal and marks of a test is the total CIE marks scored by the student.

Semester End Evaluation (SEE):

- SEE marks for the practical course are 50 Marks.
- SEE shall be conducted jointly by the two examiners of the same institute, examiners are appointed by the Head of the Institute.
- The examination schedule and names of examiners are informed to the university before the conduction of the examination. These practical examinations are to be conducted between the schedule mentioned in the academic calendar of the University.
- All laboratory experiments are to be included for practical examination.
- (Rubrics) Breakup of marks and the instructions printed on the cover page of the answer script to be strictly adhered to by the examiners. **OR** based on the course requirement evaluation rubrics shall be decided jointly by examiners.
- Students can pick one question (experiment) from the questions lot prepared by the examiners jointly.
- Evaluation of test write-up/ conduction procedure and result/viva will be conducted jointly by examiners.

General rubrics suggested for SEE are mentioned here, writeup-20%, Conduction procedure and result in -60%, Viva-voce 20% of maximum marks. SEE for practical shall be evaluated for 100 marks and scored marks shall be scaled down to 50 marks (however, based on course type, rubrics shall be decided by the examiners)

Change of experiment is allowed only once and 15% of Marks allotted to the procedure part are to be made zero.

The minimum duration of SEE is 02 hours

Suggested Learning Resources:

- FSSAI, Manual of Methods Of Analysis Of Foods: Milk And Milk Products, Food Safety And Standards Authority Of India, Ministry Of Health And Family Welfare, Government Of India, New Delhi, 2015.
- Handbook of Dairy Foods Analysis, Fidel Toldra, Leo M.L. Nollet, Routledge Tyler and Francis, 2021

e-resources

- https://archive.nptel.ac.in/courses/126/105/126105013/
- https://onlinecourses.nptel.ac.in/noc19_ag05/preview
- https://www.youtube.com/watch?v=qbVyZ2QxRAA

Date of experiment_____

Experiment 1 : Preparation of sample for Milk

Preparation of sample for Milk

1. **Milk**

Sample size required: 50 cc's or 1/3 cup. Vials with preservative are available from the lab. Milk with preservative will be stable for 7 to 10 days at 40 degrees to 70 degrees. Non preserved milk should be refrigerated immediately and transported to the lab within 24 hours.

2. **Metered Milk**

Mix metered milk according to manufacturer's specification. Pour mixed milk in to clean leak proof container. Transport to lab promptly.

3. **Bulk Tank**

Agitate tank for 5 to 10 minutes. For a single sample dip out agitated sample into clean leak proof container. For duplicate sampling, dip out into a separate container enough milk for all samples to be run. Mix sub sampled milk and pour into clean leak proof container. Transport to lab promptly.

4. **Individual Cow**

Milk cow off line in fresh cow bucket. Mix milk in sealed bucket. Pour mixed milk into clean leak proof container. Transport to lab promptly. It is important to note that hand striping the milk from the cow will not produce an accurate test result. First milk is generally higher in protein while later milk is generally higher in fat. Somatic Cell Count also fluctuates during the milking process.

5. **Containers**

Sterile glass or plastic vials are suitable for collecting the milk. Do not open the container until you are ready to collect the sample. If using a container with a cap, do not allow the cap to touch any surface.

6. **Collector Preparation**

Wash and dry hands with a clean paper towel before sampling. If possible, have someone who is not milking take the sample to avoid contamination from other cows.

7. **Udder Preparation**

Try to sample each cow before milking, to avoid contamination from the milkers, liners, etc. Prep the udder as normal, and make sure the teats are clean and dry. Using a separate sterile swab or cotton ball for each teat, swab the teat ends with alcohol. Clean the teats that are farthest from you first, then those nearest.

8. **Sample Collection**

Sample the teats nearest to you first. Discard the first few squirts from each teat before collecting into the bottle, and avoid touching the bottle with the teat ends. Hold the cap of the bottle so the inner surface is neither touching anything nor facing up. Direct milk at a nearly horizontal angle into the bottle. Try to sample an equal amount of milk from each quarter if you are not testing each quarter separately. Cap the bottle immediately after collection.

9. **Sample Storage**

Cool or freeze samples immediately and keep cool until arrival at the lab. It is best to have the samples cultured within 24 hours of collection.

10. **Bulk Tank Samples**

Take samples four to five days in a row. Agitate the tank for 5 minutes before sampling. Take samples from the top of the tank with a sterile syringe. Samples may be transferred to a sterile vial if desired. Freeze sample immediately after collection. Pack samples in ice so they stay frozen during shipment to the lab.

11. **Preparation of Sample of Milk**

Samples are received after few days of drawl and contain preservative (0.4% formalin). Warm the sample to 37- 40°C by transferring it to the beaker and keeping it in a water bath maintained at 40 - 45°C. Stir slowly for proper homogenisation. Mix sample thoroughly by pouring back into the bottle, mixing to dislodge any residual fat sticking to the sides and pour it back in the beaker. During mixing do not shake the bottle vigorously. Allow the sample to come to room temperature (26- 28°C) and withdraw immediately for analysis. If small clots or lumps are observed in the sample which cannot be dispersed, a few drops of liquor ammonia may be used during homogenisation. If even after homogenisation the sample shows lumps or clots or droplets of oil are visible suggestive of curdling /splitting of milk, the sample should be deemed unfit for analysis and rejected.

Date of experiment_____

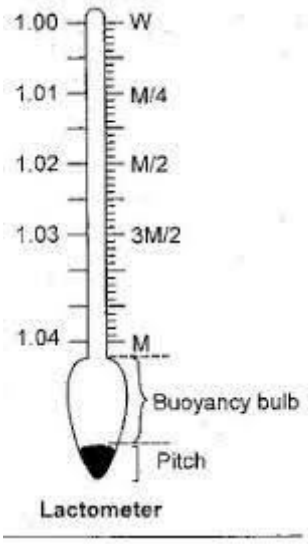
Experiment 2 Detection of adulterants in milk :

Preparation and testing of milk for adulterated water in milk using lactometer

Objective: To Check Water Adulteration in Milk by Using Lactometer

Principle:

The lactometer, employed for measuring specific gravity or density, operates on the Archimedes principle, wherein a floating object sinks until it displaces a fluid weight equivalent to its own. The density of the fluid is inversely proportional to the volume of displaced fluid, reflected in a lower lactometer reading. Richmond's formula establishes the relationship between total solids, Solids-Not-Fat (SNF) content, fat percentage, and specific gravity in milk. Normal whole milk exhibits a specific gravity of 1.029 to 1.032, contrasting with skim milk at 1.036. Milk extracted from the udder contains air bubbles, and the gradual solidification of milk fat results in a progressive volume contraction, leading to a slow rise in specific gravity—the Racknagal phenomenon. Storage duration and temperature introduce variability in milk's specific gravity. To counteract this, ensuring complete liquefaction of the fat before taking the specific gravity reading becomes crucial, a goal achieved through pre-warming the milk.



Apparatus

- Lactometer
- Thermometer
- Lactometer Jars
- Glass rods

Preparation of milk sample for analysis

- Gently warm the sample to a temperature range of 37-40°C by transferring it to a beaker and placing it in a water bath maintained at 40–45°C.
- Stir slowly to ensure proper mixing and homogenization.
- Thoroughly mix the sample by pouring it back into the bottle, stirring to dislodge any residual fat adhering to the sides, and then pouring it back into the beaker.
- Avoid vigorous shaking of the bottle during the mixing process.
- Allow the sample to return to room temperature (26-28°C) and promptly withdraw it for analysis.

- If small clots or lumps are observed in the sample that cannot be dispersed, consider using a few drops of liquor ammonia during homogenization.
- If, despite homogenization, the sample exhibits lumps, clots, or visible oil droplets indicative of curdling or splitting of milk, deem the sample unfit for analysis and reject it.

B. Determination

- Invert the sample bottle gently two or three times and then pour down the milk in the lactometer jar along its side to avoid the formation of air bubbles.
- Sufficient milk should be poured into the jar to ensure that some of it overflows when the lactometer is inserted.
- The lactometer, held by the stem, is inserted in the sample and released when it is approximately in its position of equilibrium thus avoiding wetting more than a very short length of the stem above the milk surface.
- As soon as the lactometer is at rest, the scale reading corresponding to the top of the meniscus of milk is noted.
- The lactometer jar shall be vertical and the bulb of lactometer shall not touch the side.
- It is advisable to repeat the reading after depressing the lactometer about 3 mm and allowing it to come to rest.
- Note temperature of milk immediately after taking the lactometer reading with the help of the thermometer.

Observation

SN	Parameter	
1	Temperature	30°C
2	Lactometer reading (LR)	28
3	Corrected Lactometer reading (CR)	(28 + (10 * 0.2) = 30.

Result: LR value of 28
CR value of 30

Inference: LR value of 28 or CR value of 30 and corrected indicates that the milk is not adulterated with water. Milk with LR lower than 26 or higher than 32 should both be rejected as "impure".

Note to students:

The theory behind LR value measurement is very simple. LR value is based on the concept of "density". Scientifically speaking, density is defined as mass per unit volume. Simply put, it determines how thick (or heavy) a liquid is. The density of the water is considered to be: 1 g/cm³, the density of milk is around: 1.035 g/cm³, the density of honey is 1.33 g/cm³. Therefore honey will be thicker than milk and milk will be thicker than water . Lactometers are usually calibrated such that when they are immersed in pure water at a temperature of 20°C then the lactometer will immerse completely in the water and gently sit at the bottom of the container and the LR value will be zero. When it is immersed in pure milk which is at 20 °C temperature then the Lactometer will show a reading between 26-32. A value below 26 indicates that water has been mixed into the milk and a value higher than 32 indicates that solids have been added to the milk. Milk with LR lower than 26 or higher than 32 should both be rejected as "impure". Since Lactometers are usually calibrated at 20 C temperature; therefore if the milk temperature is different than 20 C then a correction should be applied. For every one-degree temperature above 20 C add 0.2 to the LR value and for every one-degree temperature below 20 C subtract 0.2 from the LR value. For example if the milk temperature is 30 C and the observed LR value is 28; then the correct value of LR should be: 28 + (10 * 0.2) = 28.

Date of experiment_____

Experiment : 3

Estimation of starch using hand held Refractometer

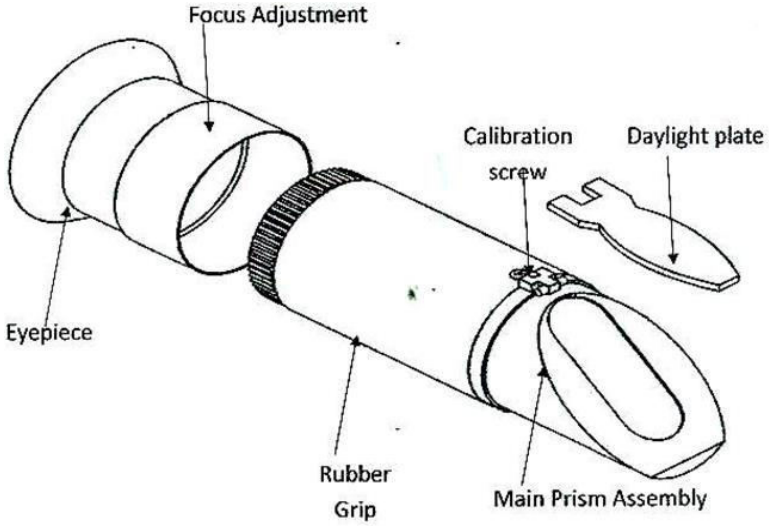
Objective: After studying and performing this experiment, you should be able to a determine TSS of milk and milk beverage.

Principle:

Total soluble solids may be determined by means of Refractometer. Brix is a measure of total soluble solids (TSS) in the case of pure sucrose solutions. Generally, milk and milk products contain more sugar than other soluble constituents, and hence, Brix provides useful guide of TSS or sugar content. The concentration of sugar solutions can be determined conveniently for routine purposes using a refractometer. There are two types of refractometers viz. hand refractometers and Abbe's refractometers, the latter being tabletop instrument, which can measure both ^{O}Brix (TSS) of sugar solutions and also there is provision for maintaining constant temperature. It should be noted that the refractometers are calibrated for milk and milk products and hence if the medium contains other soluble solutes in substantial quantities, there will be slight error. Refractometer measures total soluble solids (TSS) concentration based on the principle of refraction of light. When a ray of light travels obliquely from one medium to another, it is bent or refracted. The refraction occurs because light travels at slightly different velocities in different media, the extent being proportional to the density of the solution or the soluble solids concentration. The refractive index of a medium is defined as the ratio of the sine of the angle of incidence to the sine of the angle of refraction when a ray of monochromatic light is refracted from a vacuum (or, to a very close approximation, from air) into the medium. In a Brix refractometer, the refractive index is calibrated into ^{o}Brix readings. As refractive index is dependent on the density of the solution, the measurements must be made at a specific temperature (20^{o}C) or suitable corrections must be applied.

Equipments

- Hand Refractometer
- Thermostatically controlled water bath.
- Distilled water
- 6% solution of sucrose solution prepared freshly and stored in dark not more than 48 hours.



Procedure:

1. Calibrate the hand-held refractometer by placing few drops of distilled water on the surface of the prism.

2. Slowly secure the daylight plate and gently press the daylight plate so that the sample solution covers the entire prism surface without leaving bubbles or voids.
3. Before observing, the sample solution should be left on the prism for about 30 seconds, so that the temperature of the sample and the refractometer can be consistent.
4. Place the refractometer under the light source and observe the sample solution through the eyepiece.
5. You can see a circular area marked with a scale. If the image is not clear enough, adjust the focusing tube to make the scale line clearer. The upper part of the area is blue, and the lower part is white.
6. Observe the distilled water or standard solution through the eyepiece, and adjust the calibration screw until the boundary line of the blue and white area completely coincides with the 0 scale line.
7. It is necessary to ensure that the surrounding temperature is within the range of 20°C or 68°F, so as to ensure that the accuracy of the measurement is not affected.
8. When the temperature fluctuation of the working environment exceeds 5 degrees, please readjust to ensure the accuracy and reliability of the measurement results.
9. If the refractometer is equipped with an automatic temperature compensation system, no matter when the instrument is recalibrated, it must be ensured that the ambient temperature during adjustment is 20°C. Once the adjustment is completed, the ambient temperature can fluctuate within the allowable range from 10℃ to 30℃ without affecting the accuracy of the measurement.
10. It is necessary to replace distilled water or standard solution with the sample solution to be measured, and then repeat the first step, the second step and third step, and read the scale value of the blue and white dividing line.
11. This scale value is the accurate measurement value of the concentration of the sample solution. After use, do not clean the refractometer with water to avoid water seeping into the lens cone. It must be wiped clean with a soft damp cloth to avoid corrosion and soiling of important optical components, otherwise the measurement results will be inaccurate.

Observation

SN	**Sample**	Reding
1	Distilled water	
2	6% Sucrose Solution	
3	Test Sugar Solution	
4	Milk	

Result The readings are expressed as total soluble solids (TSS) = %.

Tips for using the refractometer

- After the measurement, you should directly wipe off the attachments on the surface of the prism and the cover with a damp cloth. After drying, it should be properly stored.
- It is a precision instrument, so be careful when using it.
- It is necessary to avoid touching or scratching the optical components.
- It needs to be placed in a dry, clean, and non-corrosive air environment to prevent the surface from blurring.
- In the process of carrying, avoid strong vibration, so as not to damage the optical components and basic structure.

Date of experiment_____

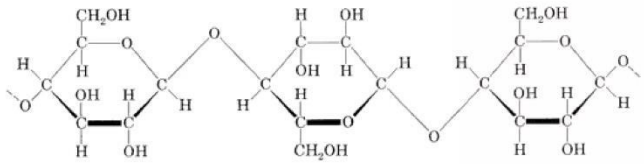
Experiment : 4

Detection of cellulose in a milk

Aim: After studying and performing this experiment, you should be able to a determine cellulosed as an adulterant in a milk.

Principle:

Cellulose is a long chain of linked sugar molecules that gives wood its remarkable strength. It is the main component of plant cell walls, and the basic building block for many textiles and for paper. Cotton is the purest natural form of cellulose. In the laboratory, ash less filter paper is a source of nearly pure cellulose.



Cellulose in milk gives blue color with Iodine – Zinc Chloride reagent.

Detection of Cellulose in Milk

Cellulose in milk gives blue colour with Iodine – Zinc Chloride reagent.

Reagent

Iodine – Zinc Chloride reagent:

- Dissolve 20 g ZnCl2 in 8.5 ml water and when cool, introduce the iodine solution (3 g potassium iodide and 1.5 g iodine in 60 ml water) drop by drop until iodine begins to precipitate.

Procedure

- Take about 10 g of milk in a 100 ml beaker.
- Add 50 ml of hot water and stir thoroughly for about 2 min.
- Pour the mixture on a nylon cloth and wash the residue with 50 ml of hot water twice.
- Scrape the residue with a spatula and place it in a spotting plate.
- Stain a part of residue with Iodine-Zinc Chloride reagent and another part with iodine solution.
- Development of blue colour in Iodine Zinc Chloride reagent and absence of blue colour in Iodine Solution confirms presence of cellulose.
- The method is also applicable to milk products like curd, rabri and evaporated milk.

Observation

Calculation

Date of experiment_____

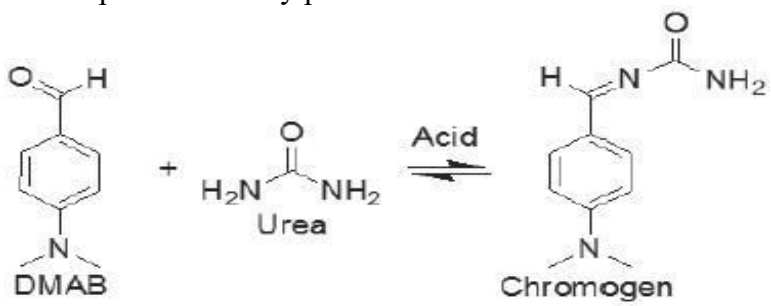
Experiment : 5

Detection of urea in a milk

Objective: After studying and performing this experiment, you should be able to a determine urea as an adulterant in a milk.

Detection of Added Urea in Milk

- Urea is a natural constituent of milk and it forms a major part of the non-protein nitrogen of milk.
- Urea concentration in milk is variable within herd. Urea content in natural milk varies from 20 mg/100 ml to 70 mg/100 ml.
- However, urea content above 70 mg/100 ml in milk indicates milk containing 'added urea'.
- The addition of urea to milk can be detected by using para-dimethylaminobenzaldehyde (DMAB).
- This method is based on the principle that urea forms a yellow complex with DMAB in a low acidic solution at room temperature.
- The reaction between p-DMAB and urea is initiated by the protonation of the dimethyl amino group that generates a charge deficiency in the carbonyl carbon, making it susceptible to a nucleophilic attack by part of the urea.



Reagents/Apparatus

- **Standard urea solution (1 mg/ml):** Dissolve 100 mg of urea (AR grade) in phosphate buffer (pH 7.0) and make up the volume to 100 ml.
- **p-Dimethyl amino benzaldehyde (DMAB) solution((1.6 per cent w/v):):** Dissolve 1.6 g DMAB in 100 ml ethyl alcohol and add 10 ml concentrate HCl. The reagent is stable for 1 month. Prepare new standard curve with each new batch of reagent.
- **Phosphate Buffer pH 7.0:** Dissolve 3.403 g anhydrous potassium dihydrogen orthophosphate (KH_2PO_4) and 4.355 g anhydrous dipotassium monohydrogen orthophosphate (K_2HPO_4) separately in 100 ml of distilled water. Combine solutions and dilute to 1 litre with water.
- **Trichloroacetic acid (TCA) 24%,** w/v: Freshly prepared. 24.0 g TCA is dissolved in distilled water and volume made up to 100 ml.
- **Diluting Reagent:** Equal volumes of 24% TCA and phosphate buffer (pH 7.0) are mixed to make the diluting reagent.
- **Spectrophotometer** – Instrument with maximum band width 2.4 nm at 420 nm, with 1 cm cells B.
- **Whatman filter paper:** Grade 42.
- Funnels.
- Test tubes.

Procedure

Preparation of standard curve

- Pipette 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 , 1.4, 1.6, 1.8 ml aliquots of standard urea solutions into 10 ml test tubes and label
- Make up the volume of all the test tubes to 5 ml by adding 5.0,4.8, 4.6, 4.4, 4.2, 4.0, 3.8, 3.6, 3.4, 3.2 using phosphate buffer.
- Add 5 ml DMAB solution to each.
- Shake tubes thoroughly and let stand for 10 minutes.
- Read Absorbance(A) in 1 cm cell at 420 nm with reagent blank at zero A.
- Plot A against concentration urea Plot should be straight line

Estimation of Ura in milk sample

- 10 ml of milk sample is mixed with 10 ml of TCA to precipitate the proteins
- Filtered using Whatman 42 filter paper.
- 5 ml of filtrate(TCA) Extract is then treated with 5 ml of the reagent to develop the colour.
- Blank is prepared by taking 5 ml of diluting reagent and treating with 5 ml of DMAB reagent.
- The optical density of the yellow colour is measured at 420 nm. From standard curve the amount of urea in milk is calculated.

Observation

	Preparation of standard curve										Test samples	
	B	1	2	3	4	5	6	7	8	9	T1	T2
Milk Sample	-	-	-	-	-	-	-	-	-	-	5	5
TCA	-	-	-	-	-	-	-	-	-	-	5	5
Filtration	-	-	-	-	-	-	-	-	-	-	Filtered using Whatman 42 filter paper Take only 5 ml for next stage.	
Volume of the Std. Urea solution or Test Sample	0.0	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8	5.0	5.0
Volume of phosphate buffer	5.0	4.8	4.6	4.4	4.2	4.0	3.8	3.6	3.4	3.2	0.0	0.0
DMAB solution	5	5	5	5	5	5	5	5	5	5	5	5
Shake tubes thoroughly and let stand for 10 minutes.												
Absorbance at 420 nm (x axis)												
Concentration of Urea in a sample mg/ml(calculated) (y axis)	0.00	0.02	0.04	0.06	0.08	1.00	1.02	1.04	1.06	1.08		

T1 is fresh milk, T2 is adulterated milk

Graph: plot standard graph of graph of y axis versus x axis

Date of experiment_____

Experiment 6: Detection of fat content in milk

Estimation of Fat content in milk by Centrifugal Separation of Fat (Volumetric Gerber Method)

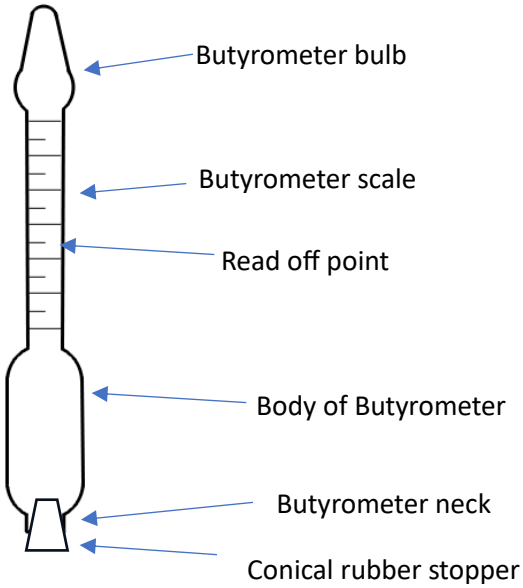
Objective:

To determine the fat content in a given sample of milk or milk product.

Principle:

The Gerber Method is a primary and historic chemical test to determine the fat content of substances, most commonly milk. The Gerber Method was developed and patented by Dr. Niklaus Gerber of Switzerland in 1891. In this method milk fat is separated from proteins by adding sulfuric acid (1.820-1.825 sp. gr. at 60°F). The separation is facilitated by using amyl alcohol and centrifugation. The fat content is read directly via a special calibrated butyrometer.

It is based on the principle of measuring the volume of fat released from a known volume of milk or known weight of product in a specially devised and accurately calibrated modified cylinder called butyrometer. After taking 10 ml of the Gerber acid (Sulphuric acid with specific gravity 1.84 at 200°C) in the butyrometer 10.75 ml of milk is added to it in such a way that it forms a layer above the acid without mixing with it. Amyl alcohol (1 ml) is added to break the emulsion. The sulphuric acid dissolves the milk fat in it after breaking the fat globule membrane through its coagulating effect on proteins. The fat dissolved in the acid is separated through centrifugal force and read in the stem of butyrometer.



Schematic diagram of butyrometer

Reagent and equipment:

- Butyrometer; with 6%, 8% and 10% scale for whole milk.
- Automatic measures or 10 ml pipette for Gerber acid.
- Automatic measure or 1 ml pipette for amyl alcohol.
- Milk pipette (volumetric) having 10.75 ml mark at 270 C.
- Gerber centrifuge capable of being rotated at 1100 rpm.
- Gerber acid (H2 SO2) specific gravity 1.84 at 270 C (90- 91% by weight)

Procedure

- Transfer 10 ml Gerber acid to milk butyrometer with automatic measuring device.
- Add 10.75ml of well-mixed sample of whole milk

- Milk is added to butyrometer slowly and carefully in such a way that the tip of the pipette touches the base of the neck of the butyrometer and form a layer above the acid without dissolving in it.
- Add 1 ml of amyl alcohol with auto measure and close the neck of the butyrometer firmly by inserting the stopper so as not to disturb the content.
- Carefully shake the content by applying gentle swirling motion.
- After thoroughly mixing the content place the butyrometer in water bath maintained at 65ºC for 5 minutes.
- After removing from the bath and drying the butyrometer along with its contents is centrifuged it for 5 min at 11000 rpm.
- Again, keep the butyrometer along with its in the water bath for 5 minutes and read the fat percentage by inverting the butyrometer and keeping gradually and at eye level.
- The reading of the butyrometer from the two mark as the scale which corresponds to lowest point of the clear fat meniscus and to the fat acid interface.

Observation

Abr.	Samples	Weight in g
W	Weight of the sample	
W1	Weight of flask and fat	
W2	Weight of flask	

Results

Fat % =((W1- W2)/ W)x100

Where:
W = Weight of the sample
W1 = Weight of flask and fat
W2 = Weight of flask

Inference

Whole milk contains approximately 3.25-3.5% milk fat or 8 grams. Lower fat milk such as 2% contains 5 grams, followed by 1% milk with 2.5 grams. Finally, there is skim or fat-free milk containing 0 grams of milk fat.

Note to students:

- The theory behind using 10.75 ml milk in the pipette is as follows:
- Gerber butyrometer is graduated on 0-10 scale and calibrated in such a way that each 1% division represents 0.125 ml of fat.
- The weight of the fat in the area is equal to Volume × Density = Mass; 0.125 x 0.9 = 0.1125 (because density of fat = 0.9 g/lit)
- If 1 % represents 0.1125 then 100 % will be represented 11.25 g.
- As per this, we should be pipetting 11.25 g of milk, but there are certain impurities due to iso-amyl alcohol, which affects the fat reading. These impurities are estimated at 2.5-3% (average (2.5+3)/2 = 2.667%).
- So the fat is 1.125-(1.125×2.667/100) = 1.095 g.
- According to this we should be pipetting 10.95 g of milk.
- This is equivalent to 10.65 ml of milk (10.95/1.02547, the denominator being the density of milk).
- Since 0.1 ml residual milk remains in the glass pipette sticking to the walls, we take 10.75 ml of milk

Date of experiment_____

Experiment 7: Detection of gelatine in mil

Aim: Detection of Gelatine in Milk added as adultrant

Principle

The milk is considered as a valuable food for all the human beings including the children and adults. The milk is rich in easily digestible nutrients and provides highly nutrient diet. The world population is increasing day by day which creates an alarming situation for the adequate supply of the milk to everyone along with the optimum quality of the product. It is a highly perishable commodity hence; it should be consumed within a definite span of the time or otherwise should be preserved with a suitable preservative.

Reagents: Mercury, Conc. HNO_3, Saturated Picric Acid solution.

Procedure

- Take 10 ml of sample, add 10 ml acid Hg(NO3)2 solution (Hg dissolved in twice its weight of HNO3 and this solution diluted to 25 times its volume with water).
- Shake mixture, add 20 ml water, shake again, let stand 5 minutes and filter.
- If much gelatine is present, filtrate will be opalescent and cannot be obtained quite clear.
- To portion of filtrate in test tube add equal volume of saturated aqueous picric acid solution.
- Yellow precipitate is produced in the presence of considerable amount of gelatine, smaller amounts are indicated by cloudiness.
- Note: The test is applicable to milk products also. In applying this test to sour, fermented, cultured, or very old samples of milk, cream or butter milk ; to sterilized cream or evaporated milk or to cottage cheese, use care to recognize precipitate produced by picric acid when added to the $Hg(NO_3)_2$ filtrates from these materials in absence of gelatine.
- Such samples with or without rennet and entirely free from gelatine, give on standing distinct precipitate when treated as above. In every case, however these precipitates differ in character than those produced by picric acid with gelatine.
- Gelatine picric acid precipitate is finely divided, more apt to remain in suspension, settles only slowly and adheres tenaciously to the bottom of the container, from which it is rinsed with difficulty.
- Precipitates produced by picric acid in the absence of gelatine are flocculent, separate readily (leaving serum practically clear) do not adhere to walls of container and are easily removed by rinsing with water.
- When gelatine is present in sample gelatine picric acid precipitate will remain in suspension long after flocculent precipitate has settled, but on standing overnight the characteristic sticky deposit will be found adhering tenaciously to bottom and sides of the test vessel.
- If gelatine is present in relatively high concentration (1%) gelatine, picric acid precipitate will be voluminous and will settle rather quickly.

Date of experiment_____

Experiment 8: Determination of pH in whey water

Objective: To determine the pH of given sample of milk or milk product.

Principle:

The term "pH" refers to the measurement of hydrogen ion activity in the solution. Since the direct measurement of the pH is very difficult, specific electrodes are needed for quick and accurate pH determination. pH is measured on a scale of 0 to 14, with lower values indicating high H+ (more acidic) and higher values indicating low H+ ion activity (less acidic). A pH of 7 is considered as neutral. Every whole unit in pH represents a ten-fold increase in or decrease in hydrogen ion concentration. Most natural waters possess the pH values ranging from 5.0 to 8.5. The pH of milk is 6(slightly acidic) while that of curd is in the range 4.5 - 5.5. The name acid whey reflects the low pH of the whey, typically ranging from 3.6-4.5 with an average value of 4.1 (Table 1). Sweet whey, a co-product from the production of hard cheeses, often has a pH of 5.6 or higher, by comparison. pH is measured using pH meter, which comprises a detecting unit consisting of a glass electrode, reference electrode, usually a calomel electrode connected by KCl Bridge to the pH sensitive glass electrode and an indicating unit which indicates the pH corresponding to the electromotive force is then detected. Before measurement, pH meter should be calibrated by using at least two buffers.

Standardisation of pH meter

- The pH meter can be standardized by measuring the buffer solution of 4, 7, and 9 or any other solution of standard pH.
- Sometimes, the manufacturer of the pH meter may suggest other methods of standardizing, which too must be followed.
- The electrodes must be inserted into the water so that it does not touch the bottom of the beaker.
- Bottom contact with damage may cause damage to the electrodes.
- Any cause of slow response due to the polarization can be solved by washing the electrodes thoroughly.
- Periodic check must be conducted to check the electrodes
- During the electrode storage, they must be kept moist.
- And also follow the instructions of the manufacturer.

EQUIPMENT REQUIRED:-

- pH meter
- pH electrode filled with KCL solution
- Buffer solutions of pH4 and pH 7
- Clean beakers
- Tissue papers
- Distilled water
- Thermometer

Procedure:-

- Plug in the pH meter to power source and let it warm up for 5 to 10 minutes.
- Wash the glass electrode with distilled water and clean slowly with a soft tissue.
- Note the temperature of water and set the same on the pH meter.
- Place the electrode in pH 7 buffer solution and set the value of 7 on the pH meter turning the Calibrate knob on the meter.
- Take out the electrode, wash with DW and clean.
- Dip the electrode in the pH 4 buffer solution. Adjust the value on the pH readout meter by the Slope switch .
- Repeat with pH 9, pH 7 and pH 4 buffers till a correct and stable reading is displaced.
- While moving and cleaning the electrode, put the selector switch on standby mode.

- Turn to pH mode for recording the pH.
- Now place the electrode in the water sample whose pH is to be determined.
- You can take a number of simultaneous readings for different samples until the power is on
- **Observation**

Abr.	Samples	Initial reading	Reading after calibration
1	pH of the standard buffer of pH 4		
2	pH of the standard buffer of pH 7		
3	pH of the standard buffer of pH 9		
4	pH of the sample		

Results: pH of the given sample is

Date: …….

Experiment 9: Determination of the Percentage of Total Solids and moisture

Aim: To determine the percentage of total solids and moisture in the given samples of milk.

Principle

Total solids determination is a common procedure in many manufacturing plants using dairy products. The total solids in milk can be calculated from the specific gravity and fat percentage from the table wherein lactometer reading is taken at 60° F. After undertaking this activity, you will be able to: explain the technique of determination of the total solids in the samples of milk and milk products, and analyze the percentage total solids in different types of milk available.

Materials Required

- Two samples of milk (Whole milk and tonned milk)
- Shallow flat bottom dishes of aluminium alloy, nickel, stainless steel, porcelain/silica about 7-8 cm in diameter and 1.5 cm in height.
- Air oven maintained at temperature 100°C
- Weighing balance

Principle

Total solids determination is a common procedure in many manufacturing plants using dairy products. The total solids in milk can be calculated from the specific gravity and fat percentage from the table wherein lactometer reading is taken at 60° F. Lactometer reading for a given percentage of fat is used to calculate the total solids in milk. Besides carrying out the total solids percentage from the indirect method of taking lactometer reading, a direct method of gravimetric analysis can also be used. This method involves accurately weighing a few grams of the material and subjecting it to heat until all moisture has been driven off on a water bath. The dry residue is weighed, its percentage calculated as total dry solids.

The knowledge of the fat content and the specific gravity of the milk can determine total solids in milk. The percentage of total solids can be measured by the application of a simple formula as given below:

$$T = 0.25\ G + 1.2\ F + 0.14$$

where,

T represents the percentage of total solids

G represents the specific gravity (in degrees)

F represents the percentage of fat

Procedure

Direct method of calculating total solids is as follows

Now carry out the experiment step-by-step as enumerated herewith:

- Weigh accurately the clean, dry empty dish with the lid.
- Pipette into the dish about 5 ml of the prepared sample of milk and weigh quickly with the lid on the dish.
- Place the dish uncovered on a boiling water bath.
- Keep the base of the dish horizontal to promote uniform drying and protect it from direct contact with the metal of the water bath.
- After at least 30 minutes remove the dish, wipe the bottom and transfer to an air oven maintained at 100°C ± 2°C.
- At the end of one hour, remove the dish to a desiccator and allow to cool completely before weighing again.

- To ensure the constancy of weight, the dish must be replaced in the air-oven for successive periods of 30 minutes, with intermediate weighing, until two consecutive weighing differ by less than one milligram.
- Note the lowest weight.
- Calculate the total solid percent by weight using the formula given herewith.

Calculations

Total solids, percent by weight $=\frac{W2-W3}{W2-W1}$X100

where,
W1= weight in g of the empty dish
W2 = weight in g of the prepared sample taken for test.
W3 = weight in g of the residue after drying.

Precautions

- The dish should be kept on a water bath for the evaporation of water.
- In the oven, do not place the dish near the walls of the oven.

Results/Findings

Record you observations/findings here in the format provided and calculate the total fat percent.

	Milk sample 1	Milk sample 2
W1= weight in g of the empty dish		
W2 = weight in g of the prepared sample taken for test.		
W2 = weight in g of the residue after drying		
Total solids, percent by weight =(W2-W3)/(W2-W1)X100		

Date: …….

Experiment 10: Preparation sample of curd condensed/falvoured milk and determination of titrable acidity.

Aim: To determine the titrable acidity of the given samples of milk**.**

Principle:

The titrable acidity test measures the amount of alkali which is required to change the pH of milk from its initial value of about 6-6 to 6.8, to the pH of the colour change of phenolphthalein added to milk to indicate the end point (pH 8.3).

Reagents

- Standard Sodium Hydroxide Solution – 0.1 N. B.
- Phenolphthalein Indicator – Dissolve 1.0 g of phenolphthalein in 100 ml of 95% ethanol. Add 0.1 N NaOH solution until one drop gives a faint pink colouration. Dilute with distilled water to 200 ml.

Procedure

- Weigh accurately about 10 g of the material in a suitable dish or basin.
- Add 30 ml of warm water.
- Add 1 ml of phenolphthalein indicator.
- Shake well and titrate against standard NaOH solution.
- Complete the titration in 20 seconds.
- Keep a blank by taking 10 g of material diluted with 30 ml of water in another dish for comparison of colour 9.5.3.

Observation

	Initial reading	Final reading	Total volume
Reading 1			
Reading 2			
Reading 3			

Calculation

Titratable acidity as Lactic acid = 9 AN/W

Where, A = Volume of standard NaOH required for titration

N = Normality of Standard NaOH solution

W = weight of the sample taken for test

Date: …….

Experiment 11: Preparation sample of dried milk and determination of protein

Aim: Analyse the milk sample in terms of its protein content

Principle:

The rapid method for determining the percent protein content in milk is the Lowrys method method which, in effect, analyzes total nitrogen. Protein nitrogen derived from amino acids represents approximately 95% of nitrogen in milk. Non-protein nitrogen, such as urea, exists in minor quantities at approximately 5%.

Equipments and reagents:

- Lowry protein Assay method:
  Solution A: 2% (w/v) sodium carbonate in 0.1 M sodium hydroxide (2mg/mL).
  Note: (1M NaoH = 40g of NaOH pellets in 1000 mL water, 0.1M NaOH = 4g of NaOH pellets in 1000 mL water) (or 16g in 400 ml)
  Solution B: 1% (w/v) copper sulphate (0.1g/10 mL water).
  Solution C: 2% (w/v) sodium potassium tartrate (0.2g/10 mL water).
  Solution D: Copper reagent- Mix 0.5 volume of solution B, 0.5 volume of solution C and 50 volumes of solution A. (4mL of B, 4 mL of C and 400 ml of A)
- **Solution E:** Folin-Ciocalteu reagent is diluted to 1M acid according to the supplier's instruction (1 vol Folin-Ciocalteau reagent diluted with 1 vols water). Prepare 5 ml.
- **Standard protein solution:** Dissolve 10mg of BSA (as it is easily available, cheap and with improved purity) in 100ml of distilled water in a volumetric flask. (for concentration-100 µg/ml)
- Spectrophotometer.

Plotting Standard Graph

- Pipette out aliquots of 0.0, 0.2, 0.4, 0.6, 0.8, and 1.0 ml of standard bovine serum albumin in six test tubes.
- Make the volume to 1 ml in every test tube by adding distilled water.
- Add 0.5 ml of alkaline copper solution (Solution D)to all the test tubes and thoroughly mix in vortex.
- Add 5 ml of FC reagent (Solution E)to all the test tubes and thoroughly mix in vortex.
- Incubate at room temperature for 30 min in dark.
- Incubate at room temperature for 10 min in dark.
- Read the od at 660 nm.
- Plot the standard graph of OD versus concentration of protein.

Lowry Method to estimate protein:

1) To 1 mL of the test solution, add 5mL of solution of Solution D (Copper reagent), mix thoroughly by vortexing and stand at room temperature for 10 min.
2) Add 0.5 mL of solution E (Folin-Ciocalteau reagent), mix rapidly, and incubate for 30 min at room temp.
3) Measure the absorbance at 600nm against reagent blank not containing protein.
4) The concentration is estimated by referring to a standard curve obtained at the same time using known concentrate of bovine serum.

Observation

	Standard curve						Test samples				
	B	1	2	3	4	5	T5	T10	T15	T20	Total protein
Volume of the BSA or Test Sample	0.0	0.2	0.4	0.6	0.8	1.0	1.0	1.0	1.0	1.0	1.0
Volume of Water in mL	1	0.8	0.6	0.4	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Volume of alkaline copper solution, Reagent D	5	5	5	5	5	5	5	5	5	5	5
Incubation at room temperature in dark– in min	10	10	10	10	10	10	10	10	10	10	10
Volume of F.C reagent, Reagent E	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Incubation at room temperature in dark– in min	30	30	30	30	30	30	30	30	30	30	30
OD at 600 nm											
Concentration of Protein in mg/mL											

Graph:

Date: …….

Experiment 12: Preparation sample of dried milk and determination of ash content

Aim: Analyse the milk sample in terms of its ash content

Principle:

The principle of ashing is to burn off the organic matter and to determine the inorganic matter remained. Heating is carried out in two stages:- firstly to remove the water present and to char the sample thoroughly; and finally ashing at 550°C in a muffle furnace. This method is applicable to all food materials. In this method (IS: 11962-1987) a test portion of the sample is incinerated at 825 ± 25°C and the weight of the residue obtained is recorded. The following method is as per IDF guidelines.

Apparatus

- Silica or platinum dish: about 70 mm diameter and 25 to 50 mm deep.
- Electrical furnace with air circulation, capable of being controlled at 825 ± 25°C.
- Desiccator: Containing an effective desiccant. 17.7.2.

Procedure

- Heat the dish in the electrical furnace, controlled at 825 ± 25°C, for 30 min. allow the dish to cool in the desiccator to the room temperature and weigh to the nearest to 0.1 mg.
- Weigh, to the nearest 0.1 mg directly in or by difference into the prepared dish, approximately 3 g of the test sample.
- Heat the dish with its content on a low flame until the test portion is completely charred, taking care that it does not burst into flame.
- Transfer the dish to the electrical furnace, controlled at 825 ± 2°C, and heat for at least 1 h until all carbon has disappeared from the dish. Allow the dish to cool in the desiccator to the room temperature and weigh to the nearest 0.1 mg.
- Repeat the operations of heating in the electrical furnace, cooling and weighing, until the mass remains constant to within 1 mg or begins to increase. Record the minimum mass.

Observation

	Weight in g
Weight of the crucible with lid = W_1	
Weight of the crucible with lid + sample = W_2	
Weight of the crucible with lid + sample = W_3	
Weight of the initial sample M_o= (W_2-W_1)	
Weight of the ash M_1= (W_3-W_1)	

Calculation

The ash of the sample, as a percentage by mass, is calculated using the following formula.

Ash content =((M_1)/M_o)*100

Where

M_o = mass in g, of the test portion.

M_1 = mass in g, of the prepared dish.

Calculate the ash to the nearest 0.01% and report the final result to the nearest 0.1%.

Date: …….

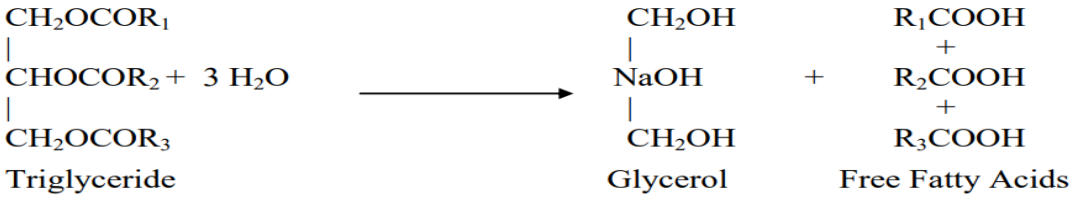
Experiment 13: Preparation sample of butter and determination of free fatty acids

Aim: Determination of Free Fatty Acids in Ghee

Principle:

The acidity (free fatty acid content) of a fat is normally a measure of the extent to which hydrolysis has liberated the fatty acids from there ester linkage with the parent glyceride molecule. Partly for this reason, acidity of ghee is extensively quoted as a free fatty acid content percent (% FFA). The FFA content of fresh ghee varies from 0.09 to 0.28% with an average of 0.16%. The sensory quality of ghee deteriorates with increase in FFA content. As per FSSAI Rules (2011), ghee should not contain FFA more than 3%. Reaction 1 Level of acidity shows the proneness of fat to oxidation. Highly acidic ghee shows faster oxidation, and thus has poor keeping quality. With the increase in acidity, there is decrease in consumer acceptability.

Reaction 1



The FFA present in ghee can be estimated by acid-base titration with alkali (NaOH) using phenolphthalein as an indicator and the end point comes at around pH 8.3 (BIS, 1966).

Reaction2

Apparatus and Reagents

- **Conical flasks:** 250 ml capacity.
- **Burette:** 50 ml, graduated to 0.1 ml.
- **Ethanol or rectified spirit:** 95% (v/v), sp. gr. 0.816, neutral to phenolphthalein.
- **Sodium hydroxide or Potassium hydroxide:** 0.1 N aqueous solution accurately standardized against oxalic acid (AR grade) or potassium phthalate.
- **Phenolphthalein indicator:** 1.0% solution in 95 % (v/v) ethanol or rectified spirit. 13.4.1

Procedure

- Weigh 10 g of the ghee sample in a 250 ml conical flask.
- In another flask bring 50 ml of ethanol to the boiling point and while still above 70°C, neutralize it to phenolphthalein (using 0.5 ml) with 0.1 N NaOH.
- Add the neutralized alcohol to flask containing ghee sample and mix the contents of the flask.
- Bring the mixture to boil and while it is still hot, titrate with 0.1 N NaOH, shaking vigorously during the titration.
- The end point of the titration is reached when the addition of single drop produces a slight, but a definite colour change persisting for at least 15 sec.

Observation

	Initial reading	Final reading	Total volume
Reading 1			
Reading 2			
Reading 3			

Calculation

- The acidity of ghee can be expressed in different ways:
- **Free fatty acids:** The acidity of ghee is frequently expressed as the percentage of free fatty acids in the sample, calculated as oleic acid, using following formula:
  Free fatty acids (as Oleic acid) = (T/ M) x 2.82
  Where,
  T = volume of 0.1 N alkali required for titration in ml;
  M = mass in g, of ghee sample taken.