A COMPREHENSIVE REVIEW ON POTENTIOMETRIC BIOSENSORS

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Abstract:
With improvements in the detection limits and selectivities of ISEs, the introduction of new materials, new sensing concepts (from conventional potentiometry to dynamic electrochemistry approaches), and deeper theoretical understanding and modelling of the potentiometric responses of ISEs, potentiometry based on ion-selective electrodes (ISEs) has experienced a renaissance. The latest developments promote advancements in ion sensing and biosensing software. Additionally, by employing ISEs as potent transducers, flexible sensing methods have been developed for a wide variety of different target molecules in response to the introduction of new bioreceptors, including as enzymes, antibodies, aptamers, and pepetides. This study examines current potentiometric biosensor trends. There have been discussions about their uses in the biosensing of metal ions, tiny molecules, DNA, proteins, microbes, and toxins.Based on the combination of potentiometric ISEs with novel materials and cutting-edge methodologies, this review offers an outlook for potentiometric biosensing.

Keywords: Ion-selective electrode, bioreceptor, potentiometric biosensing, and potentiometric biosensing
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Introduction:

The use of biosensing technologies in healthcare, industrial process control, environmental monitoring, and military applications is on the rise. Thus, much effort has been put into creating adaptable, sensitive, and selective biosensing devices for a variety of targets using transduction modes that are electrochemical, optical, and mass-sensitive. Amperometric, potentiometric, impedimetric, and conductometric approaches are some electrochemical detection techniques. A well-established analytical technique for measuring essential electrolytes physiologically is potentiometry based on polymeric membrane ion-selective electrodes (ISEs). Potentiometric sensors have the advantages of being tiny, quick to react, simple to use, inexpensive, and immune to turbid and coloured interferences. ISEs also have certain distinct characteristics. In contrast to other analytical procedures that yield the total concentration, they supply information on the free ion concentration (ion activity). Since they are, at least in theory, independent of sample volume, the detection limits are not adversely affected by a sharp reduction in sample volume. These characteristics actually distinguish ISEs from other indication electrodes or detectors. In the last 20 years, ISEs have seen a quiet revolution with the discovery of ion fluxes through the polymeric membrane of ISEs in the 1990s. Low detection limit ISEs, polyion

sensors, solid-contact ISEs, and new sensing ideas 4 for dynamic potentiometry are just a few of the remarkable advancements in ISEs that have been made.Promising models and numerical simulations for mechanisms were put out concurrently. The lower detection limit and selectivity coefficients have often improved by factors up to 106 and 1010, respectively, since the groundbreaking work on lower detection limit ISEs introduced by the group of Pretsch. Potentiometry is now one of the most sensitive electrochemical techniques available when used at trace levels in constrained samples. Significant advancements have been made in understanding the response mechanism and their applications for the detection of highly charged macromolecules in biological samples since the groups of Meyerhoff and Yang discovered the polyion-selective electrode. Applications for potentiometric sensing have recently shown promise thanks to the advent of nanopores. Most notably, Bakker's group introduced and had been adopted by researchers 5 attractive dynamic electrochemistry techniques, such as chronopotentiometry, controlled reagent release, and coulometric analysis, to build attractive methodologies. Calibration-free sensors are now possible thanks to new ion-selective readout concepts based on chronopotential, transition time, charge of transient current pulse, voltammetric current and optical signal. The use of ISEs for point-of-care diagnostics and on-site environmental analysis utilising a disposable or flexible diagnostic equipment has found additional applications in recent years. Wang's team has created a variety of tattoo-based potentiometric ion-selective sensors and wearable multi-ion potentiometric sensors using the tattoo-based platform and specially produced stretchable materials. Wireless sensor networks, such as those used for body sensor networks and distant environmental monitoring, are becoming more and more common. A new generation of platforms for healthcare, diagnostics, and environmental monitoring have been created by integrating several ISE types with wearable sensor arrays. Moreover, for places with low resources, a handheld device that connects sensors, including potentiometry, directly to "the cloud" using any mobile phone may be a good choice. At the present, scientists have recognised potentiometry based on ISE as a significant, cutting-edge technique to identify targets including ionic species, neutral species, small molecules, and biomolecules.Potentiometry has been recognised as a potent transducer for biosensing with the recent advances in ISEs. The developments in potentiometric biosensor between 2011 and 2019 are compiled in this review. Outside the purview of the current review are light-addressable potentiometric sensors and ion-sensitive field-effect transistors (ISFETs). It should be mentioned that potentiometric biosensing has benefited greatly from the advancement of biosensors based on biologically coupled ISFETs. Examples include the direct DNA sequencing of genomes and the detection of antigen-antibody responses using pH-sensitive ISFETs. The current study highlights recent developments in potentiometric biosensing applications with a focus on the use of polymeric membrane ISEs.It is shown how ISEs can be used to potentiometrically detect metal ions, tiny compounds, DNA, enzymes, proteins, bacteria cells, and toxins. It also illustrates the future of potentiometric biosensing based on the incorporation of potentiometric ISEs with novel materials and cutting-edge methodologies.

BASIC CONCEPT OF BIOSENSOR

A biosensor detects an analyte by measuring it utilisizing a biological component and transforming the signal into a detectable form (Fig.1). The transducers turn the biological stimulus into a measurable signal(Narwal et al., 2019).

Fig 1 Biosensor- Principle, components,types and their applications

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A biosensor is a self-contained integrated device, capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor), which is in direct spatial contact with a transducer element,' according to a recently proposed IUPAC definition"(Qian et al., 2017). Biosensors are widely used in diagnostics, medical, agriculture, veterinary, bacterial and viral diagnostics, medicinal manufacture, waste water treatment, military and defence applications(Pundir et al., 2018).Biosensors shows wide range capability to manifest itself in varying application. This category includes applications such as environmental monitoring, illness detection, food safety, defence, medication development, and more. One of the most common uses of biosensors is the detection of biomolecules that are either disease markers or therapeutic targets(Bhalla et al., 2016).Electrochemical biosensors amalgamate with the benefits of electrochemical techniques with the enzyme's impeccable binding with substrate, rapid feedback, and handle to use.Nanomaterials have recently been used to increase the analytical performance of electrochemical biosensors (Pundir et al., 2017). Metal oxide nanostructures are renowned for their capacity to facilitate quicker e^- transfer kinetics b/w the electrode and active sites of the targeted enzyme. With the advancement of nanotechnology in recent years, a large number of novel nanomaterials have been developed, and their novel features are increasingly being identified, as well as their applications in biosensors(Lata et al., 2012a). Diverse biosensors for detecting cholesterol levels in various biological samples have received a lot of interest in recent decades.

BIOSENSOR ADVANTAGES: Biosensors are distinguished by the following characteristics:Stability**,** Economical, Senstivity, Reproducibility(Souza et al., 2001, souza et al., 1999)

COMPONENTS OF BIOSENSORS

The biosensor's block diagram is divided into three sections: sensor, transducer, andelectric circuit.

1. Sensor or detector: "The sensors or detectors, which is a biological component, is the first part. It is a biological receptor, after all. It interacts with analyte and sends an electrical signal to indicate a change in its composition"(Darsanaki et al., 2013).
2. Transducer: "The transducer is a physical component that amplifies the biochemical signal received from the detector, converts the resulting signal to electrical, and displays it in a readable manner"(Darsanaki et al., 2013).
3. Electrical circuit: "It is a component that includes a signal conditioning unit, a processor or microcontroller, and a display".

Working of biosensor

In biosensors, "the combination of a biological sensitive element and a transducer converts biological material into an electrical response in the form of a signal. The output of the transducer will be either current or voltage, depending on the type of enzyme. Everything is in order if the output is voltage"(Narwal et al., 2019).If the output is current, it must first be converted into equivalent voltage.The low-amplitude output voltage signal is superimposed on a high-frequency noise source. As a result, before passing through a Low Pass RC Filter, the signal is amplified. The

signal processing unit's output is known as an analogue signal"(Saraju P. Mohanty., 2001). "The biological quantity being measured is comparable to this output. The analogue signal can be displayed directly on an LCD display, but it is commonly transferred to a Microcontroller, where it is transformed into a digital signal"(Huang et al.,2017)

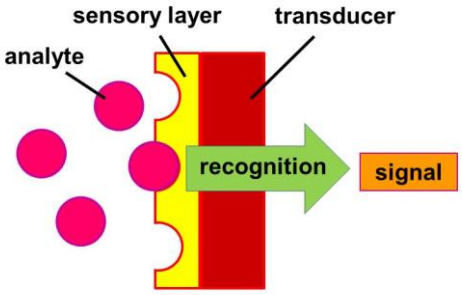

Fig. 2. Schematic figure of biosensor (Pundir et al.,2018)

Classification of cholesterol biosensor

Cholesterol biosensors can be classified using a variety of parameters such as bioreceptors, transducers, and other forms of physical and chemical interactions. Cholesterol biosensors can be categorised into the following types based on the transducer: electrochemical (amperometric and potentiometric), conductometric, optical, and piezoelectric.

Analytic methods of cholesterol determination:

Determining the quantity of cholesterol is critical in human body (Nauck et al., 2000, Staels et al., 2002, Ikonen et al., 2008). For the quantification of cholesterol levels, many biosensor techniques have been applied, including calorimetric, spectroscopic, Potentiometric and electrochemical approaches(Sperry et al., 1943, Nakaminami et al., 1997, Trettnak et al., 1993). Different methods for assessing cholesterol are currently available, including "density-gradient and sequential ultracentrifugation, chromatographic procedures, precipitation and electrophoretic techniques"(Lalla et al., 1954, Bhandaru et ,al., 1977 Chung et al., 1980, Kieft et al., 1991, Wang et al., 2002,). However, older methods for determining serum cholesterol have a number of limitations, including a lengthy, cumbersome, and labour-intensive approach, as well as lower specificity and sensitivity(Batra et al., 2021). The concept of a biosensor was suggested to circumvent these constraints(Mahato et al., 2018, Purohit et al., 2020, Haleem et al., 2021, Nagraik et al., 2021). For cholesterol determination, a variety of alternative investigative techniques have appeared, such as colorimetric(Bhandaru et al., 1977), spectro photometric (Manasterski et al., 1973), enzymic colorimetric (Mizuno et al., 1980), and electrochemical techniques (Morycki et al., 2015). Among all the famous electro analytical devices or previous last half century decades(Power et al., 2013), traditional colorimetric test methods for cholesterol measurement have a number of drawbacks, including fluctuating ester reactivity, reagent colour uncertainty, corrosive nature of the reagents, poor specificity, and lack of reagent reusability(Giri et al., 2014). To overcome these problems, the development of biosensor were purposed. A biosensor is an analytical tool that uses a biological element to analyse an analyte. and turns the signal into a detectable form. Firstly, the concept of biosensor was coined by Clark in 1950 to determine several other compounds such as cholesterol, lactate, and uric acid (Gahlaut et al., 2017). For the determination of cholesterol, numerous approaches have been developed, including "gas chromatography/mass spectrometry, thin layer chromatography, liquid chromatography, spectrophotometry, fluorimetric method, and so on"(Wang et al., 2021)(12,13). However, these approaches are time-consuming, require sophisticated processes, have low specificity, and are expensive. Aside from the general flaw of various common traditional methods, each

method has their own limitations, such as interference of many substances with the colour reaction in colorimetric methods, faint spots in thin layer chromatography, and difficult to reproduce results in thin layer chromatography(Narwal et al., 2019). As a result, there is a need of economical, simple & sensitive approach for the detection of cholesterol(Ibupoto et al., 2014). Electrochemical techniques, particularly enzyme based sensors-system have been developed, for cholesterol handling with increased interests [13]. The 1st potentiometric enzyme sensor was published in 1969(Guilbault et al., 1969). Potentiometric sensors, in general, there is a no need of an external power source & do not pass current during detection, making them ideal for creating sensors for biological systems(Bobacka et al., 2008).

WORKING MECHANISM OF CHOLESTEROL BIOSENSOR

The functioningprinciple for the cholesterol biosensor during quantification can be described by considering the double role of cholesterol oxidase(ChoX), which serves as both a specificity enhancer and a catalyst to start the chemical reaction. The Thechemical process creates 5-3-ketosteroid and hydrogen peroxide as a result of the enzyme catalytic reaction between cholesterol molecules and oxygen(o) present in the solution of electrolyte (Ibupoto et al., 2014) in Eq.(1)

$$\text{Cholesterol} + O_2 \xrightarrow{ChOx} 5\text{-3-ketosteroid} + H_2O_2 \quad \text{(Israr et al.,2011)}$$

However, the isomerization of the trans double bond 5–6 of the steroid ring with the intramolecular transfer of a proton from the 4–6 position produces 4- 3-ketosteroid as stable molecules Eq. 2.

$$5\text{-3-ketosteroid} \xrightarrow{\text{Isomerisation}} 4\text{-3-ketosteroid (Israr et al., 2011)}$$

The above hypothesised electrochemical process is supposed to be liable for the generation of charges near the working electrode's surface, resulting in a potential diff. between the cholesterol biosensor and the reference electrode within the electrolyte solution(Ibupoto et al., 2014).

Potentiometric biosensors

Potentiometry has evolved into the industry standard for the clinical study of ions, particularly for the identification of biologically important electrolytes like Na+, K+, Ca2+, and Cl-1 in physiological fluids or even in cells [3,4, 67]. For instance, Wang's team created a tattoo-based potentiometric sensor in conjunction with a miniature wearable wireless transceiver for real-time monitoring of human perspiration [68]. Many businesses now view electrolyte potentiometric sensors as mature products. Their future developments for other targets, though, look promising. The potentiometric biosensing based on the usage of bioreceptors such enzymes, antibodies, aptamers, peptides, or even whole cells will be the main emphasis of this review. We list the uses of potentiometric biosensors for metal detection in the ensuing sections.The applications of potentiometric biosensors for the detection of metal ions, small molecules, DNA, enzymes/proteins, bacteria cells, and toxicity are outlined in the sections that follow.Potentiometric biosensors transform biological reactions to electronic responses using ion-selective electrodes, pH metre glass electrode or solid states electrodes are the most often used electrodes.Biosensors detect & measure the ion or the electron produced in a variety of reactions; in this case, weak buffer solutions are to be used.The amount of gas produced is detected or measured using gas detecting electrodes.This type of biosensor measures the potential difference between two solutions separated by an ion-selective membrane with virtually little current flow(Yunus et al., 2013). The voltage at which these reactions take place denotes a specific reaction (Souza et al., 1999, Bilitewski et al., 2000, Mulchandani et al., 1998, Lei et al., 2006, Higson et al., 1994, Lazckanet al., 2007).

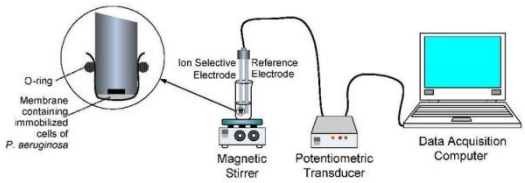

Fig 3- Experimental setup for potentiometric biosensor

2.1 Metal ions

Due to the use of organic solvents, into which protein macromolecules can only be weakly dissolved and rarely retain their functions, it is difficult to incorporate biological components into PVC membranes. An Ag+ -ISEs incorporating metallothioneins as ionophores was created using polysulfone, a porous and biocompatible polymer that can insert biological components into the membrane via a phase inversion process [69]. Unfortunately, the applicability of this approach are constrained due to the absence of biomolecules for particular metal ions. For complexations with copper ions, peptide nanofibrils were employed as biorecognition components [70]. By adjusting the peptide sequence to bind with a particular metal ion, these self-assembled peptide nanofibrils enable selective detection of metal ions. Gyurcsányi's group just recently presented a promising andGyurcsányi's team recently put up a promising and all-encompassing proposal based on hydrophilic ionophore-modified nanopores. In order to create ISEs, hydrophilic ligands such metal-binding peptides were utilised as a solid support in the form of a nanoporous membrane [71]. Moreover, some metal ions can generate metal-mediated base pairs by preferentially binding to natural or synthetic bases in DNA duplexes. For instance, Hg^{2+} has been demonstrated to exclusively bridge thymine (T) bases, whereas silver ions can preferentially coordinate cytosine (C) bases. For potentiometric biosensing of ions, the probes with mismatched bases were employed as host molecules [72]. Moreover, metal 8 specific DNAzymes were chosen and exploited as a potential platform for sensing a variety of metal ions, including DNAzymes for Pb^{2+}, Cu^{2+}, and Zn^{2+} [73]. These DNAzymes can function as bioreceptors for highly selective potentiometric detection of metal ions.

2.2 Small molecules

An ionophore with great selectivity over discriminating ions is not accessible for many small compounds. Other approaches to the detection of small molecules have been devised, such as analyte-induced enzymatic reactions or inhibition of enzyme activity, immunoassays based on antibodies or aptamers. With an enzyme electrode, numerous tiny chemicals involved in an enzymatic reaction can currently be potentiometrically detected. There are several different matrixes and strategies for immobilising enzymes. Zinc oxide (ZnO) nanostructures, which stand out among all of these matrixes thanks to their special properties including biocompatibility, high electron-transfer rate, and ease of fabrication, have generated a lot of attention in potentiometric biosensor applications [76]. Zinc oxides have been modified with enzymes for potentiometric detection of a variety of small compounds, including cholesterol [77,78], galactose [79], L-lactic acid [80], uric acid [81], and others as a suitable matrix for enzyme immobilisation.For these techniques, the ionic distribution changes around the ZnO nanostructures may be caused by enzyme processes, which could affect how the electrode reacts. Small compounds can also be found without enzyme immobilisation using two alternative strategies. One approach uses the detection of ions produced by an enzymatic reaction to measure the target molecule, which serves as an enzyme substrate. Based on this sensing concept, creatinine [82], urea [83], glucose [84,85], glutamate [86], and permethrin [87] were identified. Recently, it was shown that the bacterium Klebsiella sp. MP-6, which was isolated from soils that had been exposed to long-term organophosphorus pesticide contamination, was capable of degrading the

pesticide methyl parathion to create p-nitrophenol.Under simple conditions, the p-nitrophenol can be deprotonated, which allows the membrane electrode with an anion exchanger to detect it [88]. In the alternative protocol, the target functions as an enzyme inhibitor. The tiny molecule can be indirectly found by monitoring enzyme activity [89]. For instance, cholinesterases are permanently inhibited by organophosphate pesticides. On the basis of BuchE inhibition, a potentiometric biosensing method for the detection of organophosphate pesticides can be developed [90]. By using the precise binding between antibodies and antigens, immunoassays can be used to identify tiny compounds. However, in order to gain immunogenicity prior to immunisation, tiny compounds must be attached to carrier proteins, which may limit the range of uses.Aptamers, which are single-stranded DNA or RNA oligonucleotides chosen in vitro to bind a range of analytes from tiny ions to big proteins with high specificities and affinities, have recently come into use as an alternative to antibodies for biosensing [91]. It is envisaged that the creation of new, broadly applicable small-molecule sensing platforms would be made possible by an aptamer's capacity to convert a particular small molecule signal into the output of a potentiometric signal. An aptamer-doped gelatin layer was created by Nagels and colleagues for potentiometric sensing of the kinetics of small molecule/biomolecule interaction. Surface potential caused by target binding was used as a signal [92].Qin's team created a potentiometric aptasensor based on the target-binding driven variation of the surface charge by layer-by-layer assembling carboxylated multiwall carbon nanotubes, poly(diallyldimethylammonium chloride) (polycation), and aptamer (polyanion) on an electrode surface (negative to positive). The addition of polyions can significantly alter the charge on the electrode surface, increasing the sensor's sensitivity [93]. To signal aptamer/target binding events, Ding et al. created a label- and substrate-free potentiometric platform [94]. For adenosine triphosphate, potentiometric titrations or the direct detection based on chronopotentiometry were established (ATP). Although being elegant, this protamine-based detection method is only applicable to aptamer/target pairs that can cause a high enough conformational change, which is a difficult requirement in many cases.A universal, sensitive, simple, and label-free potentiometric test based on metal-mediated DNA base pairs for ATP was additionally developed by the same group [72]. In this approach, a nucleic acid was created that had cytosine-rich sequences in the lateral parts and the target binding motif in the centre. By preventing the creation of the metal-mediated DNA base pairs, a target binding-induced aptamer conformational change alters the concentration of silver ions at the interface of silver ISEs, which may be detected potentiometrically. Ding and his colleagues only recently established a sensitive and adaptable potentiometric platform that enables potentiometric sensing to be used to any kind of molecule [95]. In this technique, an aptamer is used to incorporate DNA nanostructures onto magnetic beads during a hybridization chain reaction (HCR). The bisphenol-aptamerThe disintegration of the DNA nanostructures caused by a binding event causes a significant alteration in the surface charge of the magnetic beads. Protamine can be used as an indication by a polycation-sensitive membrane electrode to detect such a change in surface charge. A wide variety of targets can be easily detected with this technology since aptamer can recognise a variety of targets. In spite of the flexibility and adaptability of potentiometric aptasensing of small molecules, choosing aptamers with high binding affinities remains difficult.

Cholesterol potentiometric biosensor

A “Potentiometric cholesterol” determination electrochemical biosensor based on ZnOnanorods is proposed. Using a low temp.aqueous chemical method, hexagon-shaped ZnOnanorods were directly grown on a silver wire with a diameter of 250 m. Physical adsorption of cholesterol oxidase (ChOx) onto ZnOnanorods was used to

immobilise it. The electrochemical response of the ChOx/ZnO/Ag biosensor was studied as a log.function of cholesterol conc. (1×10^{6} M to 1×10^{2} M) against a standard reference electrode (Ag/AgCl), revealing good linearity and a sensitivity of 35.2mV per decade (Israr et al., 2010)

The stabilised polymeric lipid membrane was immobilised over a graphene electrode to create a novel potentiometric cholesterol biosensor. The ChOx enzyme and polymerization mixture make up the stabilised polymeric lipid membrane, which has a significant impact on the cholesterol biosensor's properties. With a linear slope curve of 64 mV per decade, the given biosensor demonstrates good repeatability, selectivity, and sensing capabilities. Because stabilised polymeric lipid membranes and human biofluids are biocompatible, they can be used for genuine blood samples and other biological purposes (Nikoleli et al., 2013)

"The introduction of new materials, new sensing concepts (from conventional potentiometry to dynamic electrochemistry approaches), and a deeper theoretical understanding and modelling of the potentiometric responses of ISEs, potentiometry based on ion-selective electrodes (ISEs) has been injected with new vigour and gone through a renaissance"(Nikoleli et al., 2017). Ion sensing and biosensing applications will benefit from the new breakthroughs.Furthermore, with the advent of new bioreceptors such as enzymes, antibodies, aptamers, and peptides, flexible sensing methods for a wide range of target molecules have been developed using ISEs as potent transducers. The current state of potentiometric biosensors is discussed in this publication. Metal ions, tiny molecules, DNA, proteins, microbes, and toxins have all been discussed as biosensors. Based on the combination of potentiometric ISEs with novel materials and upcoming methodologies, this review discusses the future of potentiometric biosensing (Ding et al., 2020)

By electrostatic conjugation with cholesterol oxidase, "chemically fabricated zinc oxide ZnOnanowalls on aluminium wire were studied and used to create a potentiometric cholesterol biosensor. The sensitivity, specificity, reusability, and stability of the conjugated surface of ZnOnanowalls with a thickness of ~80 nm were studied at logarithmic concentrations of cholesterol electrolyte solution ranging from 1×10^{-6} to 1×10^{-3} M. The presented biosensor has a good linear sensitivity slope curve of ~ 53 mV/decade, which corresponds to cholesterol concentrations," and a 5s output response time (Israr et al., 2011)

Cholesterol biosensors that are very sensitive, selective, dependable, and affordable are in high demand for frequent monitoring of cholesterol molecules in order to prevent heart failure. Co_3O_4 nanostructures are created utilising a low temperature aqueous chemical growth approach with polyvinyl pyrrolidone surfactant as a growth template. Scanning electron microscopy and X-ray diffraction techniques were used to explore the morphology of nanostructures. The nanostructures have a morphology of interconnected nanowires, similar to a network of interconnected nanowires. Co_3O_4 has polycrystalline nanostructures.For chemical sensing of cholesterol molecules, the cholesterol oxidase was physically adsorbed on the linked nanowires of Co_3O_4. With a sensitivity of 94.031 mV/decade, the sensor device identified a wide range of cholesterol concentrations from 1×10^{-7} M to 1×10^{-3} M. A detecting limit of 0.035×10^{-7} M cholesterol concentration was discovered, as well as a quick response time of 10 seconds. The gadget that was created is extremely stable, selective, sensitive, repeatable, and reproducible. All of the information gathered regarding the given cholesterol biosensor points to its possible use in monitoring cholesterol concentrations in human blood serum and real-world samples (Ibupoto et al., 2014). Depending on the crystallinity, quality, and surface charge of the materials, several methods such as cross-linking(Tamiya et al., 1990), covalent bonding(Rajesh et al., 2005), and entrapment(Singh et al., 2004, Wang et al., 1999) are employed to immobilise the ChOx enzyme.

THE ROLE OF IMMOBILIZATION ENZYME IN CHOLESTEROL BIOSENSOR

Membranes are thought to be an excellent supporter of enzyme immobilisation. They are simple to move and mechanically durable, and they are inexpensive. Because membranes are microporousin nature, chemical modification is required to immobilise enzymes (Gahlaut et al., 2017).Immobilized enzymes are enzymes that have been physically contained or localised in a particular area of space without affecting their catalytic activity, allowing them to be employed repeatedly and continuously. It will improve enzyme specificity (kcat/Km) while also lowering product inhibition[21]. It also has a high affinity for the proteins &toprovides reactive functional groups for chemical modifications, making it ideal for enzyme immobilisation [33]. Non-conducting matrices, in addition to conducting polymers, are appropriate for enzyme immobilisation. PVC is an insulating matrix. It's also been utilised to keep cholesterol hydrolysing enzymes immobilised. HRP was inserted in a carbon electrode, and cholesterol oxidase and Cholesterol esterase were covalently immobilised on the surface of a PVC beaker that served as a reaction chamber[37]. Immobilization improves the enzyme's operational stability. For immobilising enzymes, the concept of stabilisation has been a major driving force [22].. Enzyme immobilisation on the electrode surface allows for reusability and lowers enzyme preparation costs. The method of enzyme immobilisation is the most important factor in determining the biosensor's sensitivity and stability [23].

IMMOBILIZATION TECHNIQUES.

Enzyme can be immobilized in different types of methods:

1.Physical adsorption

Physical adsorption is the most basic method of reversible immobilisation based on weak type of forces such as hydrophobic-interactions, van-der Wall forces, ionic and H-bond. Because the strength of the interaction is influenced by pH, salt conc. and temp.the interaction can be reversed by altering the solvent's pH, ionic strength, temp., or polarity. With physical adsorption, enzyme fall off is a common problem due to weak interactions [24].The enzyme is connected to the matrix by hydrogen bonds or various charge attractions in the adsorption process. Adsorption immobilises negatively charged horseradish peroxidase in a multilayer of negatively charged poly(2,6-pyridinedicarboxylic acid) in the first layer and positively charged poly(allylamine hydrochloride) in the second layer (Yang et al., 2004).

2. Covalent bonding

The most stable method of immobilising enzymes is through the formation of covalent bonds. Covalent coupling can be accomplished by activating the matrix with an activated group can be created by adding a reactive functional grp.to a polymer. The advantage of covalent bonding is the stability of the bonds formed around theenzy.and matrix, as well as the quick or suddenreaction as well as a reduction in enzyme leakage.However, thechemical changes can cause denaturation of enzymes by altering their 3D conformation [25].

3. Crosslinking

There was only one method ofenzy.immobilisationthats do not require a support is crosslinking. Cross-linking occurs when polyfunctional chemicals such as “glutaraldehyde($C_5H_8O_2$), diazonium salt, hexamethylenedisocyanate, and N–N' ethylene bismaleimide” form covalent bonds between the molecules of an enzyme. Though the crosslinking method of immobilisation that is less expensive, does necessitate the use of a purer enzyme. Because it only consumes a small amount of immobilised enzyme, it requires a high level of activity. The use of polyfunctional reagents also has the disadvantage of denatureing the enzyme and lowering its activity [25,26]. To join the enzyme with the substrate in the cross-linking technique, a cross-linking agent such as glutaraldehyde(Tamiya et al., 1990) is required. Covalent attachment, on the other hand, involves the formation of chemical bonds.

4. Matrix Entrapment

When low-molecular-wt. substrates and the products that are involved in the process, entrapment of enzy.within gels or fibres is the best option. Entrapment methods entail encasing an enzyme within a polymeric network that allows substrate and products to pass through while keeping the enzymeintraction. However, for gel formation enzyme entrapment necessitates derivatization of enzy. free residues, that are may impair enzyme activity[25].Electropolymerization in one step using conducting polymers like polypyrrole(Singh et al., 2004, Brahim et al., 2001) and polyaniline (Wang et al., 1999) is a rapid, convenient, and common approach for entrapment. The enzyme is imprisoned inside the polymer during the entrapment process, and the thickness can be changed arbitrarily by adjusting the polymerization duration.

The covalent attachment and cross-linking approaches outperform better to the others in terms of immobilising performance, which could be due to the presence of strong attractive forces created by chemical bonds(Nein et al ., 2009).

Table 1- A comparison properties of various cholesterol biosensors

Sr. no	Types of electrode	Linear range	Detection limit	Potential	Sensitivity	Time	pH/Precision	Storage/stability	Reference
1.	ChoX/Znonanorods/AgCl	1 * 10^{-6} to 1 * 10^{-6}	-		35.2Mv	10 sec	-	_	Israr et al.,2010
2.	ChoX/Graphene/ AgCl/PBS	-	10^{-7}M		64mV	5 sec	7.0	_	Nikoleli et al., 2013
3.	ChoX/nanowires CO_3O_4	1x10^{-7}M to1x 10^{-3}M	5 x10^{-8}M		94.031 mV	10 sec	7.3	_	Ibupoto et al., 2014
4.	ChoX/Lipid Film/Zno/AgCl	1x 10^{-6} M to 1 x 10^{-3} M	4 x10^{-7}M		57mV	5 sec	-	_	Psychoyios et al.,2012
5.	ChoX/ CA-CNT	10^{-3} to 10^{-8}M	10^{-8} M		-	-		_	
6.	ChoX/ Znonanowall/ Al	1 x10^{-6} to 1 x 10^{-3} M	-		53mV	5 sec		_	Israr et al., 2011

Type oftransducer /Support forimmobilization	WorkingElectrode	Method ofimmobilization	OptimumpH	Linear range(Mm /l)	DetectionLimit (mM)	Response Time(sec.)	Sensitivity(mV/decade)	Interference	Storage stabilityat4°C(days)	Ref.
Nanowires	NR	NR	NR	5 X 10 4	5 X 10 4	NR	NR	NR	NR	Syedetal 2010
Poly urethane Membrane	NR	NR	NR	NR	NR	8	NR	NR	NR	Eunazadxcicheck andexicbakker etal 2018
Poly urethane Membrane	NR	NR	NR	10-4.2 10-1.1 M	10-4.9	NR	NR	NP	1	marc parilla et al 2018
Poly urethane Membrane	Pt	NR	2.3	NR	NR	NR	79.7	NR	21	jun ho srim et al 2012
Pb 2+ ion Selectivemembrane	Pb	NR	4.7	3 X 10 -53X 10 -5	3 X 10 -5	57.4	NR		NR	pargel et al 2001
Ca sersing membrane	Ca	NR	Ne	10 -9-10 -5	NR	NR	30	NR	NR	bhakta vatsalam et al 2006
Nitrite ion selectivemembrane	NR	NR	NR	NR	NR	120	NR	NR	NR	abshax et al 2015
Sulphone membrane	NR	NR	NR	5 X 10 -61 X 10 4	1 X 10 -6	NR	NR	NR	NR	geng et al 2015
BPA	NR	NR	NR	3.2 X 10 -81 X 10 -6	1 X 10 -8	NR	NR	NR	NR	ding et al 2012

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Polymermembrane	Na+	NR	NR	NR	6 X 10 3	NR	NR	NR	NR	thuexex et al 2007
Znonanotubes	NR	NR	NR	NR	NR	10	13.17	NR	NR	zhupoto et al 2012
Zno nanotubes	NR	NR	NR	NR	NR	3	23.15	NR	NR	zhupoto et al 2013
Beads	NH 4+	NR	NR	5 X 10 -5 1.5 X 10 -3	NR	275 -465	NR	NR	NR	pundir et al 2013
Polycn – butyeacryeate	NR	entrapment	7	2.31 X 10 -3 8.25 X 10 -5	NR	NR	59.67	na+, k+ ca 2 + mg 2 + nh 4+	140	saeedfax et al 2013
Poly vinyl	NR	NR	NR	2 X 10 -5 45 X 10 -3	5 X 10 -5	NR	NR	ascarbic acia uric and amino acid	NR	kang li et al 2011
Sodium molybdate	Iodide	NR	NR	1 X 10 -11 X 10 -6	NR	NR	NR	NR	NR	karakus et al 2013
Geutamate	NH 4+	NR	NR	NR	NR	NR	NR	NR	145	
PVC	NR	NR	8.2	10 -1 – 10 - 4	NR	NR	NR	na+, k+ ca +, ascabic acid		yiemaz et al 2011
Polymeric ieacidmembrane	NR	NR	NR	NR	.1	NR	NR	NR	NR	wu et al 2009
Photocurable acxylicmatrix	NR	NR	8	NR	NR	30	56.10	NR	NR	nasix et al 2013

Cd 2+ sensitive polymer membrane	ca	NR	NR	1 X 10 -7 1 X 10 -6	NR	NR	NR	NR	NR	namnwam et al 2012
PVC	Ag	NR	NR	NR	NR	NR	59.2	NR	NR	lan et al 2014
Nanolibrics	Au	NR	NR	NR	NR	NR	NR	NR	NR	viguiex et al 2011
Polymeric membrane	Ag	NR	NR	NR	NR	NR	NR	NR	NR	ding et al 2013
Chitosan	Ag	NR	NR	NR	NR	NR	NR	NR	21	chey et al 2012
Znonanowalls	NR	NR	NR	1 X 10 -6 -1 X 10 -3	NR	NR	53	NR	NR	israr et al 2011
Lipidailm	NR	NR	NR	NR	NR	NR	57	NR	NR	psychoyious et al 2012
Znonanorods	NR	cross -linkage	NR	NR	NR	NR	41.33	asccerbic acid, urea,geulose ay 2+	NR	ibupoto et al 2012
Nabion	Ag	NR	NR	NR	NR	NR	66	ascorbic geulose urea	NR	syed et al 2012
Magneticbeads	NR	NR	NR	1 X 10 -6- 1 X 10 4	NR	NR	NR	NR	NR	ding et al 2015

Table 2 :- A comparison of potentionetric biosensors is summarized in table.

SCOPE AND CONCLUSION:

Biosensors will be widely employed in medical, agriculture, biotechnological process control, and other fields in the future. Cholesterol biosensors are a significant tool in the clinical diagnosis of a wide range of disorders. Then, there is a need to design effective software for cholesterol monitoring. Existing gadgets are not vable and do not allow for real-time cholesterol monitoring.

Patients are unable to utilise them at home. Taking these disadvantages into account, it is necessary to build low-cost tiny cholesterol biosensors. Future cholesterol biosensor research will focus on fully autonomous devices that may be readily handled by patients at home.. This considerable research has resulted in a number of technologies for electrochemical analysis of blood cholesterol, but miniaturisation of these biosensors is still being considered, so there is still a long way to go before these biosensors are available to the general public. The level of soph-istication, costeffectiveness, dependability and mobility of cholesterol biosensors will determine their appeal in the near future.Wearable and wire-free smart mobile equipment will transfer biomedical data to qualified healthcare specialists in the future, allowing them to better diagnose health condition through a non-invasive technique and personalise treatment to the specific patient's needs.This sort of automatic equipment has a number of advantages, Quick response, reduced sample input, and cost effectiveness are just a few of the benefits. A cholesterol biosensor based on enzymes has been developed, to make the manufacture process easier and more effective.

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