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Review

Chitosan in electrochemical (bio)sensors: nanostructuring and methods of synthesis

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ABSTRACT

Chitosan (Chit) is one of the most available natural biopolymer that have been extensively studied for wide range of applications such as biomedical, drug delivery, energy, environmental remediation and food industry. In this review, we have collected and discussed research literature of recent years dealing with modification/functionalization of Chit at the nanoscale and techniques of synthesis of Chit-based materials for application in electrochemical (bio)sensors. Chit-based nanomaterials functionalization strategies and the state-of-art of their incorporation to advance electrochemical (bio)sensors in various fields of applications have been thoroughly discussed together with the most effective methods of Chit deposition (molecular imprinting, layer-by-layer, and electrodeposition) and the role of Chit in biosensing promotion.

1. Introduction

Chitosan (Chit) is a polysaccharide derived mainly from chitin (poly-*N*-acetyl-D-glucosamine), a structural component of the exoskeleton of animals belonging to the phylum *Arthropoda*, and the second most abundant natural biopolymer in nature together with cellulose [1]. The name "Chitosan" refers to a group of binary heteropolysaccharides consisting of β-1 → 4-linked glucosamine and *N*-acetyl-glucosamine units, present in different fractions and patterns, with an almost Bernoullian or more block-wise distribution along the polymer chain. Homogeneous or heterogeneous deacetylation of chitin under alkaline conditions produces Chit with a varying degree of *N*-acetyl-glucosamines, which is generally expressed as percentage or fraction [2]. Chemically, *N*-acetyl-glucosamines are important for different aspects, especially for the solubility of Chit at neutral pH. On the other hand, glucosamines are important for the reactivity of Chit, for example towards oppositely charged macromolecules (e.g. hyaluronate or alginate) or small molecules (e.g. tripolyphosphate) [3,4]. Under acidic pH conditions, the amino group of glucosamine at C2 position is protonated ($-NH_3^+$) and this leads to the ionic gelation of Chit. Interestingly, in addition to this ionic gelation, Chit can also gel without external cross-linking agents [5–7] or by covalent cross-linking [8,9]. These properties of Chit make it technologically important for various applications, especially in the biomedical field, in the water treatment and in the food industry. In addition, Chit can be exploited to produce various biomaterials that can be used in tissue engineering and for drug delivery. From a biological point of view, Chit is low-toxic, non-immunogenic and biodegradable polysaccharides. Due to the presence of protonatable $-NH_2$ groups, Chit exhibits a polycationic nature and the presence of positive charges is useful to promote its antibacterial properties [10] as these positively charges form electrostatic interactions with the negative wall of the bacterial cell [11].

In recent years, great interest has been expressed in the development and integration of functional nanomaterials for the advancement of (bio)sensors. Advanced nanomaterials have many advantages, such as large specific surface area, high catalytic activity, strong electron transfer capability and excellent adsorption capacity [12].

Many strategies have been reported to incorporate Chit molecules

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into electrochemical (bio)sensors. However, due to the increasing prevalence of nanomaterials, the surface functionalization and modification of Chit is still growing and limited. This review article introduces Chit and focuses on its functionalization strategies and emerging nanomaterials (mainly graphene-based) that have been used in various fields of electrochemical (bio)sensors to improve overall analytical performance. Several recent papers highlighted Chit functionalization using biochemical strategies such as antigen–antibody and aptamers-modified Chit nanomaterials [13–15]. In this review, we first provide a brief overview of the properties and general applications of Chit, followed by a discussion of the modifications/functionalization of Chit to improve sensing capability based on the recently reported literature. In the second part, we discuss the technique of fabrication of Chit-based in the fields of sensors, chemosensors and biosensors. We have highlighted aspects and challenges to determine the optimal operational performance, such as the selection of suitable materials and the design of the geometry of all components. Thereafter, we reviewed more specifically techniques of Chit deposition in (bio)sensors including molecular imprinting (MIP), layer by layer (LBL) and cathodic electrochemical deposition (CED). In the final part of this review, we cover the state-of-art of Chit incorporation to advanced electrochemical (bio)sensors in various fields of (bio)sensors and environmental monitoring applications.

2. Chitosan — Overview

2.1. Chemical and biological properties

Chit is an interesting biopolymer as it is derived from natural sources that are abundant in nature. Chitin cannot be used directly for certain applications, especially in the biomedical field, due to its solubility problems. However, its derivative, i.e. Chit, dissolves easily in water and is also highly soluble in organic solvents. As a copolymer, Chit comprises a series of binary heteropolysaccharides composed of β-(1 → 4)-linked 2-acetamido-2-deoxy-β-D-glucopyranose (GlcNAc) and 2-amino-2-deoxy-β-D-glucopyranose ($GlcNH_2$) sugar units [1]. The *N*-acetyl-glucosamine sugar linked to glucosamine *via* glycosidic bonds are shown in Fig. 1.

Chemically, the homogeneous or heterogeneous deacetylation of chitin under alkaline conditions leads to Chit with a varying degree of acetylation (DA), also defined as the fraction of acetylated units (F_A), in the range of 0–0.7 [1]. On the other hand, fully deacetylated Chit can be homogeneously re-*N*-acetylated to form partially acetylated Chit with tunable values of F_A [3–5]. The degree of acetylation or the proportion of acetylated units is crucial for modulating the solubility of Chit at neutral pH. While all Chit are soluble under acidic pH conditions regardless of F_A and molecular weight, neutral solubility can only be achieved with certain F_A and chain lengths. More specifically, medium to high molecular weight Chit start to become soluble at neutral pH when the F_A is in the range of 0.4–0.7. In contrast, Chit with the same molecular weight and $F_A < 0.3$ are not soluble at neutral pH. In terms of molecular weight, reducing the chain length of fully deacetylated Chit is a smart approach to improve solubility of fully deacetylated Chit at neutral pH. Indeed, Chit with $F_A \sim 0$ and a very low molecular weight, which can be estimated from the number of linked sugar monomers, i.e. the degree of polymerisation (DP), typically < 10, are easily soluble up to pH = 8. Compared to other natural polymers, Chit proves to be very useful for medical applications due to its $–NH_2$ groups. The protonation of the amino groups can easily dissolve Chit in water and in acidic medium, giving Chit a positive charge as well as gelling and membrane-forming properties. The presence of $–NH_2$ and –OH groups also creates a hydrophilic environment for the biomolecules [16]. Nonetheless, Chit have been recently used to develop smart biomaterials with different sizes and morphologies that can be used in the biomedical field [9,17,18].

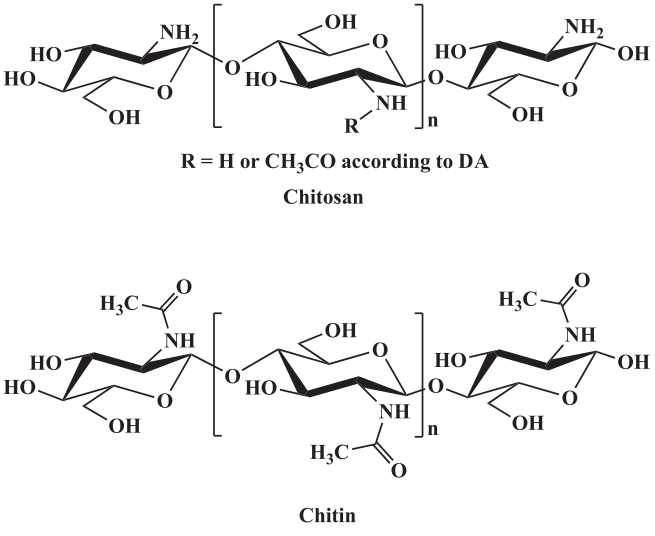

Fig. 1. Chemical Structures of Chit and chitin (DA: degree of acetylation).

From a biological point of view, it is widely recognized that Chit are less toxic, non-immunogenic and environmentally friendly polysaccharides. In recent years, the biological properties of Chit have been extensively studied. Other studies [8,19] have shown that by playing with the molecular weight, F_A and polymer concentration of Chit, its antimicrobial activities can be modulated. There is an electrostatic interaction between the $–NH_3^+$ groups of Chit and the negative cell wall of bacteria, which are responsible for the antimicrobial properties [10]. It has also been shown that the antifungal activity of Chit depends on its molecular weight [8].

2.2. Advantages of Chit in biosensing

According to origin, scientists have studied Chit's compositional structure with its chemical and physical properties. The characterization of functional Chit in its different forms is one of the hot areas of research for material scientists. The interesting noble properties of Chit and its derivatives have many applications in different field of science, discussed in literature [20], that spread from water treatment, to the food industry, cosmetics, agriculture, energy conversion [21] and biomedicine [22].

In recent years, various developments in the field of (bio)sensors have given rise to an increasing curiosity in the field thanks to the beneficial properties of biosensors which is an analytical tool. Biosensors are of low cost, portable, easy to use compared to already existing fully established other diagnostic equipment and devices. Still researchers are trying to build long-lasting, cost effective, implantable, multifunctional biosensors. Polymer based composite and nanocomposites can take these challenges, therefore researchers are working with polymer-based biosensors. Chit is known as a functional material and offers many features which are required for its utilization in different research fields. More specifically, it offers several advantages when used in electrochemical biosensors, being an excellent choice for sensor development and performance due to its unique properties resumed in Table 1.

Such properties of Chit are being exploited in different kind of electrochemical biosensors eg. potentiometers sensors and amperometric sensors (Fig. 2) for the detection of various components, as ascorbic acid, phenols, acetaminophen, H_2O_2 glucose and ethanol for a variety of applications, as soil monitoring, disease detection and food quality control [28].

The role of Chit in biosensing has been also developed in terms of promotion of the performance. Chit used as promoter functional material enhances the sensing properties by modifying nanomaterials with significant effect on the detection capability, as better explained in the next sections. The role of Chit in biosensors is also related to its ability to assemble enzyme electrostatically onto electrode surface due to its

Table 1
Advantages of Chit as electrode material in electrochemical biosensors.

Chit advantages for biosensing	Explanation
Conductivity	Chit can act as a mediator for electron transfer in electrochemical biosensors because enhances the electrical conductivity of the electrode, especially when combined with other conductive materials such as carbon nanotubes, graphene, or metal nanoparticles [23]
Film Formation	Chit can be easily formed into thin films, coatings, or hydrogels, providing a stable and consistent layer on the sensor surface for immobilizing biomolecules such as enzymes, antibodies, or DNA [24]
Functionalization	Chit can be chemically modified to introduce specific functional groups that aid in the immobilization of target molecules or recognition elements, which is crucial for the specificity and sensitivity of electrochemical biosensors [1,12]
Immobilization of Biomolecules	Chit provides a suitable matrix for the immobilization of biomolecules such as enzymes, antibodies, and nucleic acids due to its high adsorption capacity and ability to form stable complexes. This enhances the stability and sensitivity of the biosensor [25]
Porosity	Chit-based films can have a porous structure that facilitates the diffusion of analytes to the sensor's active sites, improving the sensor's response time and sensitivity [26]
Antibacterial Properties	Chit's inherent antibacterial properties help prevent contamination and prolong the lifespan of the sensor, especially when used in applications involving biological fluids [9–11]
Biocompatibility	Chit is biocompatible and non-toxic, making it safe for use in applications where the sensor may come into contact with living tissues or biological samples [27]
Cost-Effectiveness	Chit is relatively inexpensive and widely available, making it a cost-effective material for electrochemical biosensor development [1]
Stability and Durability	Chit contributes to the overall stability and durability of the biosensor, ensuring consistent and reliable performance over time
pH Sensitivity	Chit's properties can change with pH, which can be an advantage for developing pH-sensitive biosensors [16]

positive charge which helps th enzyme bonding with the electrode interface [29]. Enzymes are essential for many types of biosensors as they can catalyze specific reactions with target analytes.

The assembling ability of Chit is related to the presence of amino and hydroxyl groups, which allow the chemical enzyme immobilization at the sensor surface and helps maintain the enzymes' structural integrity and activity over time, enhancing the sensor's stability and longevity [30]. Chit provides (i) a hydrophilic environment that supports the function of enzymes, (ii) high adsorption capacity allows for a greater loading of enzymes on the sensor surface improving sensitivity and response, (iii) thin films with a porous structure, allowing for efficient diffusion of analytes to the immobilized enzymes [31].

3. Chit functionalization/modification and nanostructuring

3.1. Chit functionalization and modification

Recent advances in the design of chemo- and biosensors combining nanomaterials, molecular recognition elements and interfacial chemistry techniques have led to the fabrication of sensors that overcome challenges such as sensitivity, selectivity, stability, biocompatibility, overall electrochemical and spectroscopic performance, etc. On the other hand, the ability of functional materials, including chit-based ones, to undergo versatile modifications offers a great opportunity to further improve their properties. Recent advances in the design of chemo- and biosensors combining nanomaterials, molecular recognition elements and interfacial chemistry techniques have led to the fabrication of sensors that overcome challenges such as sensitivity, selectivity, stability, biocompatibility, overall electrochemical and spectroscopic performance, etc. On the other hand, the ability of functional materials, including chit-based ones, to undergo versatile modifications offers a great opportunity to further improve their properties. A variety of carbon-based materials (e.g., graphene (GR) [32–34], graphene oxide (GO) [35], carbon nanotubes (CNTs) [36], polymers [37], metals and metal (hydro)oxides [38], supramolecular [39]) with outstanding physicochemical, mechanical, and thermal properties and with different structures and functionalities have been introduced into chit chemistry. Therefore, a series of functionalization and modification strategies have been applied to construct and integrate various chit-based hybrid (nano) materials for electrochemical chemo/biosensors. In this section, we describe the functionalization strategies for chit-based nanomaterials and the state of the art of their incorporation to improve electrochemical (bio)sensors in different application areas. A schematic overview can be found in Fig. 3.

3.1.1. Approaches on functionalization/modification of Chit

Chit has been modified into multi-functional materials to meet requirements of (bio)sensors and overcome challenges in real-world applications. For instance, many methods were reported to produce Chit coatings scaffolds such as electrophoretic deposition, sol–gel methods, dip coating, spin coating, electro-spinning etc. [40], but it is difficult to accomplish good synergy between the nanomaterials and the Chit. On

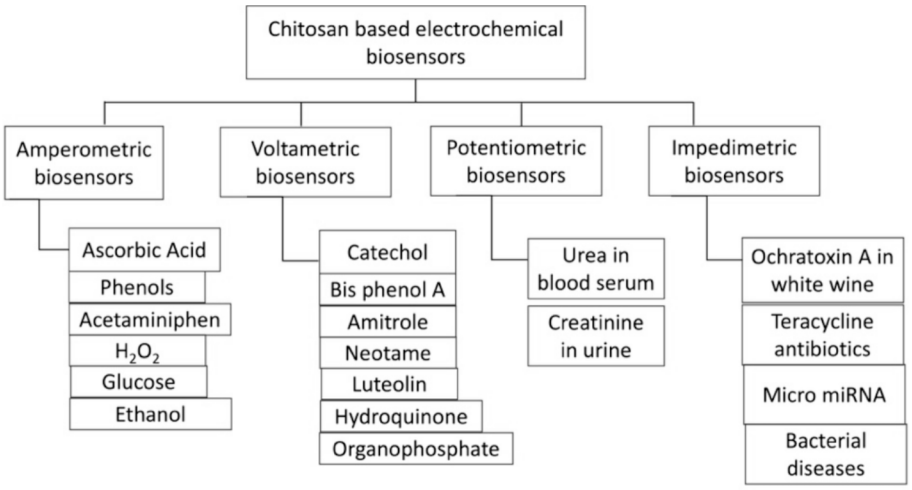

Fig. 2. Types of Chit-based electrochemical biosensors to detect different species and molecules .

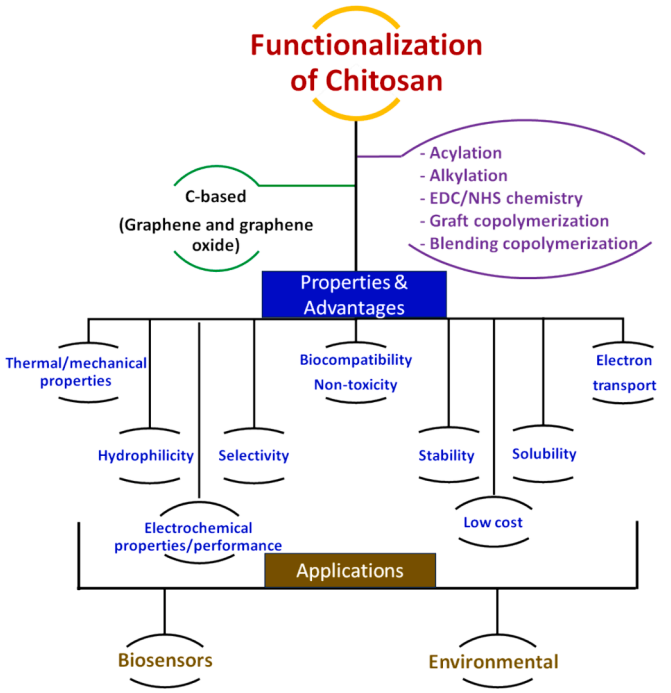

Fig. 3. An overview schematic representation of the Chit functionalization and modifications for (bio)sensors applications.

the other hand, due to the presence of the amino ($–NH_2$) and the primary and the secondary hydroxyl (–OH) functionalities on the Chit chains, with the influence of molecular weight a variety of Chit derivatives were reported. Important functionalization methods used to prepare Chit derivatives, with enhanced properties, e.g. biocompatibility, bioactivities, wound healing, solubility, and antioxidant activity, includes Chit acylation, carboxymethylation, esterification, etherification, crosslinking, physical mixing, and graft copolymerization. N-acylated Chit have been of interest among Chit's derivatives because of their ease of derivatization from a reaction between Chit and succinic anhydride [41]. To achieve biodegradable compounds with high compatibility with blood, *N*-acylated Chit is required. Acetic, propionic, n-butyric, n-valeric and n-hexanoic anhydrides (carboxylic anhydrides) was used to acylate Chit. Almost moderate degrees of N-acylation, lower than 50 %, were obtained without occurrence of gelation [42].

Nano materials functionalization with Chit is a useful technique to modify materials for potential applications. Using acylation, halloysite nanotube (HNTs) were functionalized on the surface of Chit and poly (vinyl alcohol) to utilize it in biomedical application with special emphasis on tissue engineering [43]. Produced nanocomposites were found to be biocompatible with NIH-3T3, fibroblast cells of mouse. A small amount of HNTs functionalization has proven good adhesion property as well as excellent proliferation. Bhattarai et. al. [44] and Abrica-González et al. [45], reported N-acylated Chit functionalized gold nanoparticles (AuNPs) for applications in physiological conditions and as DNA carriers, respectively. Chit is an efficient material for reduction and stabilizing of gold nanoparticles at required shape and size which depends on many physical parameters as temperature, time of reaction, molecular weight and deacetylation degree of Chit [46]. In cell culture, AuNPs functionalized Chit, acylated Chit as well as Chit oligosaccharide can evaluate transfection competence of plasmid DNA. The evaluation of different Chit-AuNP-based nanocomplexes for DNA transfection, including green/one-pot synthesis with 5 kDa Chit oligosaccharide, were of 27 %, 33 %, and 60 %, respectively.

Another approach to modify Chit derivatives is through the *O*- and *N*-carboxymethylation. In this approach, a specific attention must be paid to the solubility which especially depends on the pH. Controlling the reaction conditions such as temperature and stoichiometry will produce a rational control of *O*- and *N*-carboxymethylation yields. Other chemical functionalization and modification approaches of Chit that are widely reported in the literature are the etherification, which allows to carry out graft copolymerization. Biological properties of Chit can be improved by esterification at C-6 position where there is an hydroxyl group. It helps to make Chit more soluble, non-toxic and improves its biocompatibility [47]. Introducing of aldehyde or ketone groups along the polysaccharide chain, in which these groups can act as macromolecular crosslinking agents, has also been extensively used. The $–NH_2$ groups of Chit can efficiently react with the carbonyl groups, and forms the respective Chit Schiff base, with imine characteristic group (−RC = N-) [48]. This approach helps to increase the positively charged ions of Chit, altering its molecular structure, and improves its hydrophilicity compared to the unmodified Chit [49].

Chit is also commonly modified by methods of blending and graft copolymerization. In the former, two or more polymers are mixed with Chit by physical means, while in the latter, the polymer is covalently bonded to Chit chain or the backbone (1-deoxylactit-1-yl Chit)by chemical methods [22]. Chemical modification in Chit macromolecular chain is due to the inhomogeneous substitutional reactions which are possible at accessible sites mostly [50]. Therefore, the development of procedures for performing homogeneous chemical reactions for surface functionalization is challenging. Chit has been combined with synthetic polymers to allow better accessibility of reactive sites and to improve its processability, as well as its physicochemical properties. Chemical grafting is an appropriate and effective approach to link the polymer chains along the Chit backbone *via* its amino or hydroxyl groups. Chit-graft-poly(L-lactide) (CS-g-PLLA) co-polymers has been found to be more mechanically stable and highly reactive with biomolecules, was synthesized *via* binding PLLA chains with the backbone of Chit [51]. One terminal carboxylic acid group (PLLA-COOH) functionalization of PLLA was done by polymerization of LLA *via* ring-opening [52]. The presence of more Chit content in CS-g-PLLA copolymer increases its degradation rate. Furthermore, higher Chit amount in copolymer increases the Young's modulus with decreasing its hardness. Generally, Chit-based films become brittle which is a big drawback and make it unable to use it. In order reduce these effects and to obtain biocompatible Chit, the films were modified and subsequently incorporated by poly(ethylene glycol) (PEG) and the Chit's surface was grafted by poly(N-isopropylacrylamide) (PNIPAM) polymer [53]. The results indicated improved surface hydrophilicity and thermo-sensitivity properties of the functionalized PNIPAM-CS blend, which makes PNIPAM-grafted Chit surfaces in the development of smart biomaterials.

Enzymatic sensors are known with their high sensitivity and accuracy for detection of various analytes in different applications. However, they suffer from the drawback of stability due to the promotion of covalent and non-covalent processes resulted from poor physicochemical stability of the enzyme. For example, detection of the target heavy metals and pesticide might decrease enzyme activity due to contamination. Therefore, evolution of non-enzymatic sensors is crucial for analytes detection under harsh conditions such as high temperature, ionic strength, etc. A non-enzymatic electrochemical sensor was designed as Chit-grafted polyaniline (Chit-g-PANI)-based electrode and their detection performance towards malathion (MLT: Diethyl 2-[(dimethoxy-phosphorothioyl) sulfanyl] butanedioate) has been studied [54]. This leads to the formation of porous matrix with large redox-active adsorption at the electrode surface. Therefore, the S-atoms of MLT interact with the electrode and electrons transfer to the Chit-g-PANI at the interface, inducing potential for quantitative measurement of MLT. Moreover, the expansion of the matrix after grafting due to the biocompatibility, porosity, and long chain nature of Chit, offers efficient conduction and adsorption of MLT. A GCE immobilized with Chit@rGO-polyaniline (PAni)/ferritin (Frt)/GOx bioelectrode (as shown in Fig. 4) was developed by Haque S.U. et al. [55]. RGO-PANI composite was dispersed into the solution of Chit to increase the life expectancy storage

of the immobilized GOx based bioanode. Deacylated Chit with high degree (> 85%) is usually preferred because it offers better stability, biocompatibility, as well as less prone to degradation for the implantable glucose-based EFCs. A stable current response of 3.5 ± 0.02 $mAcm^{-2}$ is shown by used bioelectrodes in 20 mM glucose. The calculated coverage of enzyme at the Chit@rGO-PAni/Frt electrode surface was 3.80×10^{-8} mol cm^{-2} with a geometrical surface area of 0.07 cm^2. The excellent properties (such as biostability, environmentally safe, high electrical conductivity, and high surface area) offer by all components of this ternary composite make it potentially utilized for EFCs application.

As explained in more detail in section 4.2.3, electrodeposition is a simple and fast method of applying the Chit film to the electrode surface and controlling its stability with the electrode. Using a one-step deposition technique, the Chit bio-composite film can be easily used to fabricate electrochemical biosensors by integrating it with conductive materials, e.g. Au nanoparticles (NPs) [56], multi-walled carbon nanotubes (MWNTs) [57] and ionic liquid [58], an electron mediator (e.g. ferrocene) [59] and a redox enzyme (e.g. glucose oxidase (GOD)) [59]. The amino groups on Chit molecules can be conjugated to some specific elements by biofunctionalized AuNPs or functionalized biomolecules.

A conventional strategy from the 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)/N-hydroxy succinimide (NHS) crosslinking was developed and widely used to form a covalent crosslinker, to couple carboxyl groups to primary amines, which is convenient to use and is relatively inexpensive [60]. Graphene derivatives have attracted significant attention for bio-applications. However, toxicity concerns may rise due to the reactivity of epoxide moieties in the GO family [61]. Therefore, it is required to convert epoxide into carboxyl which can reduce toxicity issue. Gholamiet. al. [62] synthesized derivatives of GO nanosheets (nGO) by functionalization of nGO with Chit (nGO-CS) which is safer as well as easy to incorporate within biomaterials. Using the EDC/NHS crosslinking chemistry, nGO and Chit (low molecular weight) could attach with covalent bonding *via* an amidation process. This work claimed that toxicity of nGO was found tobe more than nGO-CS, also nGO-CS was found to be highly cytocompatible than nGO.

3.1.2. *Chit emerging nanomaterials/graphene-based*

The surface modifications of electrochemical sensors (including Chit-based) may include polymers, dyes, and enzymes; however, these may have disadvantages of handling difficulties. Recent trend is to employ nanomaterials and emerging nanomaterials like graphene, CNTs, supramolecular structures, metals and metal (hydro)oxide nanoparticles, metal organic frameworks (MOFs), etc., to overcome these difficulties, additionally, to enhance physicochemical properties, improve selectivity, sensitivity, stability, and lower the cost of the sensor, as well as overall electrochemical (bio)sensors performance. Recent years have shown great advancement of functional nanostructured materials and several recent comprehensive reviews describing the Chit-based nanomaterials for several applications [63–65]. In this section, we highlight and review Chit-based graphene nanomaterials, their functionalization, the intrinsic properties gained after integration of the functional nanomaterials, hence could be added to any other (nano)material, and their applications for electrochemical (bio)sensors.

Graphene has attracted great interests for applications in many fields including sensors, electrochemistry, energy storage, bioremediation and medical diagnosis devices due to an extraordinary electronic and mechanical properties [66,67]. As discussed above, the wide range of structural and functional advantages of Chit may provide a synergistic augmentation and expected to enhance electron transport and electrochemical properties of Chit/C-based nanomaterials including grapheme [68]. Liu et al. modified the surface of monolithic 3D graphene foam enzymatically and fabricated biosensors using it.

The biocomposite film based on enzyme, glucose oxidase (GOD), and single-walled carbon nanotubes (SWNTs)-doped ferrocene (Fc)-grafted-Chit, (GOD/SWNTs/Fc-Chit) was immobilized with 3D graphene electrode through one-step electrochemical deposition [69]. In this case, electrochemically deposited Chit biocomposite film offers a favorable biological microenvironment, and the SWNTs-doped Fc-Chit matrix improved the conductivity of the film through facilitating the electron transfer and acting as a nanowire. The procedure of one-step electrodeposition and functionalization of reagentless enzymatic biosensor is shown in Fig. 5.

An effort to detect changes in the DNA sequence using electrochemical detection process with a Chit-modified graphene platform (ChitMG) was reported by S. Alwarappan et al [70]. First, graphene was chemically synthesized and then it was covalently modified to Chit facilitating the immobilization of a single-stranded DNA 'capture' oligonucleotide. The modified ChitMG electrodes revealed a significant enhancement of the redox peak current compared with the non-modified (i.e., non-complementary mutant DNAs) ChitMG electrodes. This represents a novel, a simple, a sensitive, and a stable platform with the capability to distinguish between the complementary and noncomplementary DNA for detecting specific mutations electrochemically. Xueliang Niu et al., constructed a new electrochemical DNA sensor based on Chit-Graphene (Chit-GR) for the detection of *Staphylococcus aureus* nucgene sequence and Au nanoparticle modified electrodes [71]. In the first step, gold nanoparticles (AuNPs) were synthesized directly on carbon ionic liquid electrode (CILE) by electrodeposition. Then Chit-GR was drop-casted on the surface of AuNPs/CILE to produce Chit-GR/AuNPs/CILE nanocomposite. The modified electrode (Chit-GR/AuNPs/CILE) and due to the synergistic effects of its components demonstrated excellent electrochemical performances resulted from the proper immobilization of probe ssDNA sequence on the electrode surface through electrostatic attraction. The electrochemical performance of the

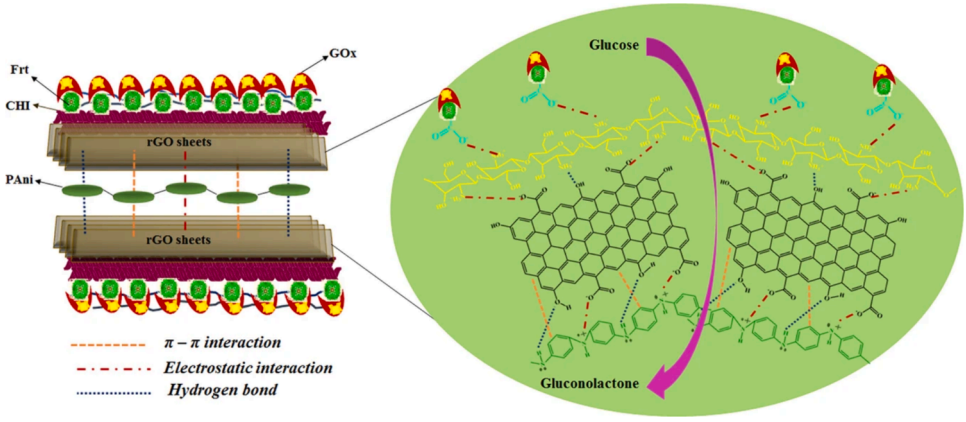

Fig. 4. Illustration of various possible interactions in Frt and Gox composite. Reprinted from [55] with permission under the terms of the Creative Commons CC BY license.

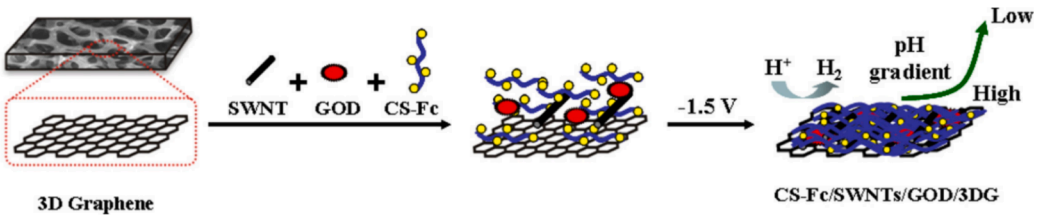

Fig. 5. Reagentless enzymatic biosensors: fabrication method using electrodeposition of Chit. Reprinted with permission from [69] Copyright 2014 American Chemical Society.

sensor, assessed with methylene blue as electrochemical indicator, was found to be proportional to the amount of the specific nucgene sequences from 1.0×10^{-13} to 1.0×10^{-6} mol L^{-1} and with a LOD of 3.33×10^{-14} mol L^{-1}. These results demonstrate the novelty of electrochemical DNA sensor to selectively distinguish mismatched ssDNA sequences.

Chit is rich in amino groups and can be converted into positively charged polymer by protonation of $-NH_2$ in acidic solutions, which may attract target analytes and pollutants such as sulfite (SO_3^{2-}) and nitrite (NO_2^-) and increases the current. Inspiring by this, a graphene oxide-Chit (GO-Chit) composite film was drop-coated onto the surface of GCE followed by gold nanoparticles layer using electrodepositing. The as-prepared simple electrochemical sensor, GO-Chit-AuNPs/GCE, was then employed for detection of NO_2^- (Fig. 6) and demonstrated great electrochemical catalytic activity towards NO_2^- [72]. Examples of other Chit-based graphene and nanocomposites with their modification and role of Chit characteristics, as well as their applications, are presented in Table 2.

4. Chit in electrochemical (bio)sensors

4.1. Principles

Electrochemical sensors and biosensors are a class of chemical sensors in which the analytical signal is generated by an electrochemical reaction with the analyte on the surface of the sensor. Depending on the functional principle, various electrochemical sensors can be manufactured, i.e. the transducer converts the electrochemical process into a measurable electrical signal, which can be amperometric, potentiometric, impedance and conductometric [88]. Selecting suitable materials and designing the geometry of all components to determine optimal operating performance is a challenge for scientists. Small variations in sensor design can greatly affect the accuracy, response time, sensitivity, selectivity and lifetime of the sensor. The material detected as well as the environment in which the sensor is used will affect the lifetime of an electrochemical sensor. In general, a life expectancy of one to three years is specified.

Among different examples of biosensors, Chit with glucose oxidase (GOx) enzymatic sensors which detect glucose. These sensors are mostly amperometric which includes the presence of a redox active enzyme. Glucose is converted into its oxidized form by the enzymes (GOx in this example), attached at the surface of electrodes. In such sensors, electrode surface detects the consumed oxygen amount and produced H_2O_2 in the enzymatic reaction, which are directly proportional to the amount of glucose present in the sensor.

Generally, in operation of electrochemical (bio)sensors a small power is required [89]. Indeed, they require minimum energy consumption among all types of sensors available for gas monitoring. Therefore, these sensors are easily used in portable devices which may contain multiple sensors.

4.1.1. Amperometric/potentiometric sensors

The amperometric technique is particularly suitable for the collection of analytes in a stream, including in vivo blood analysis of patients, or as a selective tool for the rapid analysis of a particular analyte. The portability of amperometric sensors makes them suitable detectors for field analysis [90]. The basic principle of these sensors is electron transfer between the analyte and the electrode.

The quality of the sensor largely depends on the type of cathode used, its material and shape, which is why special attention is paid to this part of the sensor. In addition, the miniaturization of the cathode to dimensions of no more than 2 μm has made it possible to develop sensors that work in vivo and in vitro in small devices, and biosensors have been developed. Beleno Cabarcas et al [91], for example, synthesized a nanocomposite based on chit and applied it to the surface of a glassy carbon electrode (GCE). With these modified GCEs, the produced amperometric biosensor demonstrated better detection capability for the direct organophosphorus pesticides. Taking advantage of the electrodeposition of chit-bio nanocomposites, which can be considered as a very attractive strategy for electrode modification, enzyme immobilization and biofunctionalization, the electrochemical performance shows a high, direct and selective determination of paraoxon with a sensitivity of 33.1nA L μmol-1, an extended dynamic concentration range of up to 40 μmol/L and a LOD of 20 nmol/L. Similarly, Lu et al [92] reported an effective amperometric sensor based on molybdate (VI) anions immobilized in a chit-modified GCE (Fig. 7) for the detection of phosphate ions in drinking water. The study was carried out using square-wave voltammetry. The high hydration enthalpy is due to the structure of the inorganic orthophosphate ions, which cannot be reduced in electrochemical reactions and therefore cannot be easily detected. This type of amperometric sensor has successfully solved the problem of

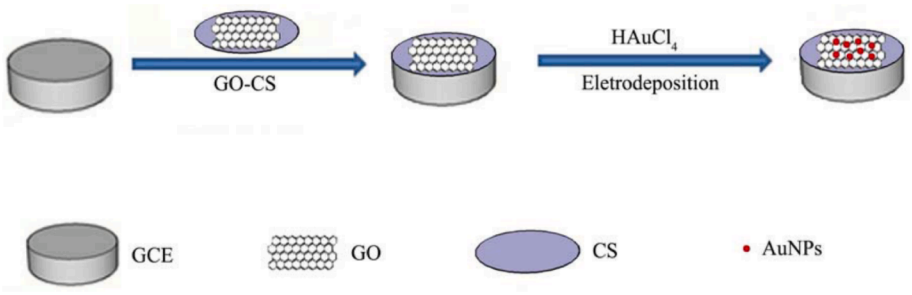

Fig. 6. A schematic representation of the fabrication steps of GO-Chit-AuNPs/GCE electrochemical sensor for detection of nitrite (NO_2^-). Reprinted from [72] with permission under the terms and conditions of the Creative Commons Attribution (CC BY) license.

Table 2
Chit-based graphene (nano)materials with their method of modification, characteristics, and electrochemical application.

(Nano)material/ Composite	Functionalization / Method of Chit (role)	Application	Ref.
Cellulose acetate (CA)-CS	Blending	Biosensing (detection of Glucose)	[73]
Graphene Oxide (GO)/CS	Covalent (EDC/NHS)	Medicinal/ pharmaceutical (Protein Nanocarriers)	[74]
CS-graphite (GR) composite	Grafting	Biosensing (detection of Dopamine)	[75]
AuNPs/CS@N,S co-doped MWCNTS) composite	Crosslinking (acetic acid activation)	Environmental (detection of Catechol and Nitrite)	[76]
Cu-hemin MOFs/ CS-reduced GO nanocomposite	A reduction and functionalized agent	Biosensing (detection of H_2O_2)	[77]
GR-CS/AuNPs	Crosslinking (acetic acid)	Environmental (determination of SO_3^{2-} and NO_2^-)	[78]
Graphene (G) and CS films	Layer-by-layer (LBL) adsorption	Electrochemical sensors (determination of dopamine (DA) and uric acid (UA))	[79]
Carboxyl graphene (CG)/polypyrrole (PPy)/CS	Simple mixing with sonication	Environmental (electrochemical NO_2^- detection)	[80]
CS-dispersed graphene nano-flakes	Chit is used as protector and disperser	For the direct electron transfer of Cytochrome *c* and biosensing	[81]
GO decorated with Ag–Ru/CSNPs	Hybrid core–shell (Chit) NPs	Electrochemical immunosensing for the detection of *Listeria monocytogenes* (Lm)	[82]
CS–vanillin (CSVan) /carbon black composites	Graft copolymerization	Energy storage (biobased polymer electrodes)	[83]
Auelectrode/ (CS–PtNPs)/ GR–AuNPs nanocomposites	Deacetylation (acetic acid)	Electrochemical molecularly imprinted sensor for the detection of erythromycin	[84]
PEDOT/PSS/CS	Electrochemical polymerization of PEDOT/PSS/CS coatings on Pt	Biosensing (detection of dexamethasone (Dex))	[85]
AuNPs-CS/G paste	Direct mixing (matrix)	Aptasensor (detection of activated protein C)	[86]
Fe-CS composite film	Electrochemical co-deposition of Fe and Chit	As(III) detection in mining wastewater/soil	[87]

phosphate detection and offers promising practical applications in water environments.

Potentiometric sensors are relatively simple, low-cost and compact, easy to integrate with electronics, thus a useful technology for micro-devices, such as biosensors. A potentiometric glucose sensor was fabricated by modifying Au coated glass substrate with iron ferrite (Fe_3O_4) nanoparticles/Chit composite which is finally immobilized by glucose oxidase enzyme. The presence of Fe_3O_4 nanoparticles and Chit offer favorable features of the magnetic properties of inorganic iron in addition to the organic Chit properties as discussed above. The potentiometric response of the fabricated glucose sensor revealed sensitivity of 27.3 ± 0.8 mV/decade in the range of 1.0–300 μM towards glucose molecules [93]. Solid-state ion sensors are one of the most and best commonly used potentiometric sensors. In particular, the ion sensitive field effect transistors (ISFETs) sensors employed for the detection of the ions (e.g. K^+, Na^+, NH_4^+, Co^{2+}, SO_4^{2-}) concentration in the solution. While the concentration of ions varies there is a corresponding current variation across the transistor [94]. The gate of the ISFET incorporates an ion selective membrane in the form of either a glass or ionophore mixture [95].

Conventional ion-selective electrodes (ISEs), with a liquid junction, suffer from the drift in the electrochemical potential. All-solid-state ion-selective electrodes (ASS-ISEs) with solid contact (SC), used as a transducer, sandwiched between the electrode and the IS film presented high value of capacitance and conductance which improve reproducibility and stability.

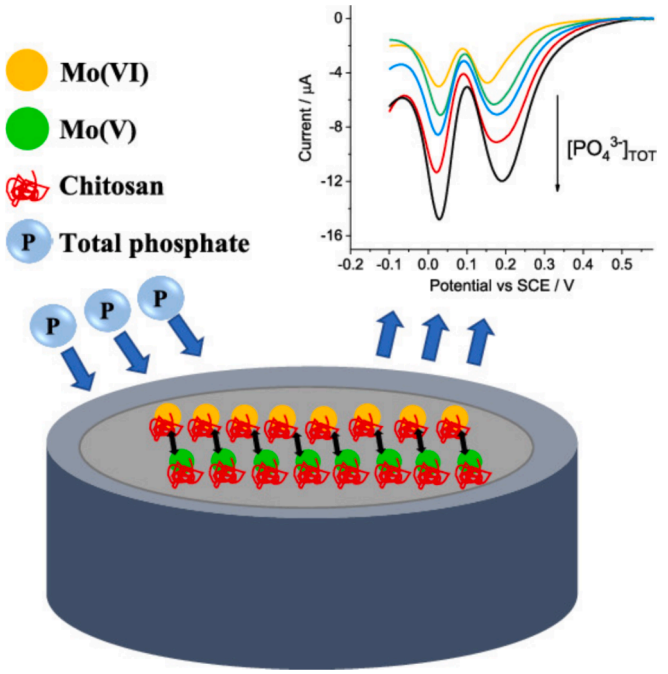

Fig. 7. A novel amperometric sensor-based on the reductive square wave voltammetry of Mo(VI) anions immobilized within a CS matrix deposited on a GCE for detection of aqueous phosphate ions in freshwater systems. Reprinted with permission from [92]. Copyright 2021 American Chemical Society.

On the other hand, these sensors may suffer from the reversible binding of interfering ions to the IS membrane, resulting in an uncertain measurement of the ion concentration that interferes with the response of potentiometric sensors [96]. Therefore, much attention should be paid to the design and assembly of SS-ISEs due to their tremendous features such as easy operation, easy miniaturization and low cost [97]. A chit/prussian blue nanocomposite (ChPBN) was chemically prepared and cast on a screen-printed carbon electrode (SPE) and served as a solid contact layer for a SS-Na + -ISE as a potentiometric Na + sensor (SPE/ ChPBN/Na + ISE) (Fig. 8) [98].

The ChPBN thin film membrane was positioned between SPE and a Na^+-IS membrane which significantly enhanced the performance due to producing high redox capacitance and fast charge transfer. The response stability shows that the ChPBN is an advisable composite as a SC in ISEs and it is comparable to materials such as colloid-imprinted mesoporous (CIM) carbon with an excellent potential drift of (3.6×10^{-4} μV/s).

4.2. Chemosensors

Research and development in chemosensors are finding more interests towards technologies for monitoring the target matrix meeting the critical needs such as simplicity, easy-to-use, and portability. Depending on the functional complexity and the functional needs, a system can be assembled by employing multi sensors with varying capabilities[99]. There is still an interesting attempt for using Chit in hybrid materials in electrochemical sensing applications in particular when a non-enzymatic sensing platform is utilized. Several reviews have recently discussed these aspects and reported somewhere else [15,100,101]. Owing to its unique properties, Chit was selected as sensing material and the sensing performance of Chit film was investigated upon hexanal gas exposure [102]. A simple approach for fabrication of an electrochemical sensor for Cu^{2+} determination in aqueous

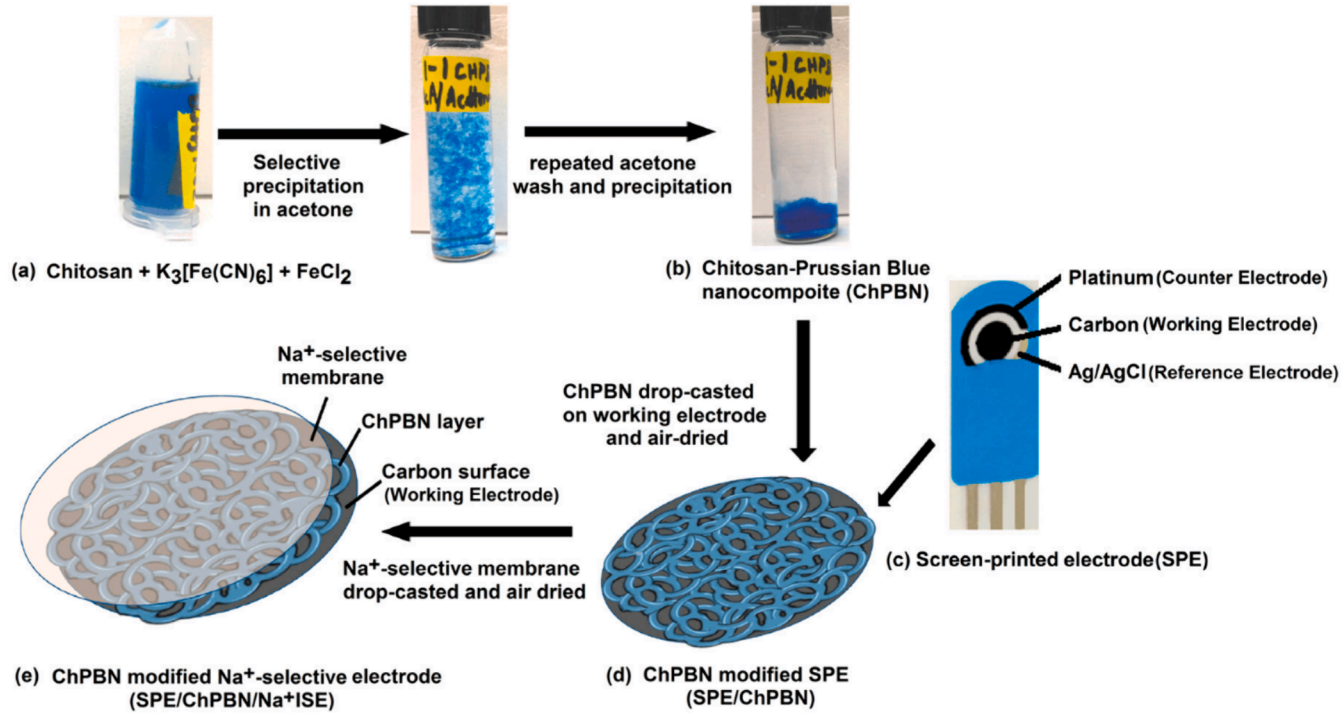

Fig. 8. Schematic representation of all all-solid-state sodium ion-sensing electrode (SPE/ChPBN/Na^+ISE): (a) synthesis of ChPBN; (b) working screen-printed electrode (SPE); (c) drop casted ChPBN solution on the SPE to fabricate SPE/ChPBN; (d) SPE/ChPBN/Na^+ISE membrane (e), ChPBN modified electrode. under the Creative Commons Attribution License.
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solution is based on hydroxyapatite nano-rods/Chit (HA/CS) nanocomposite coated on GCE surface was reported by V. Tran et al. [103]. Indeed, bare GCE can be used for determination of metal ions such as Cu^{2+} concentration but this approach suffers from poor sensitivity. Therefore, functionalized GCE surfaces, as discussed above, by polymers, inorganic compounds, or composites to generate a modified layer with abundant functionalities, which are highly attracted towards Cu^{2+} ion, such as $–NH_2$, –NH-, –OH, −SH or –COOH are very essential in terms of improving the sensitivity and selectivity for Mn^+ detection. In this coated layer, Chit with amino ($–NH_2$) groups can selectively capture Cu^{2+} ions *via* complexation interactions leading to accumulate Cu^{2+} ions onto electrodes surface for sensitive electrochemical measurement. Nanoparticles support with other properties, such as porosity, the improvement of electrochemical activity and hence the enhancement of the sensitivity for Cu^{2+} ion detection.

Chit is also used to disperse nanomaterials on solid GCE substrates. For example, nanoparticles including nano-MnO_2/Au was dispersed into Chit to modify GCE had been fabricated for amperometric determination of dopamine (DA) at a very low working potential (0.00 V vs. SCE) [104]. The modified electrode displays good amperometric response to DA in a wide concentration range 0.10 μM to 1.10 mM.

4.3. Biosensors

The development of biosensors by incorporating functional proteins, nucleic acids, specific ligands and living cells is associated with the development of methods for immobilization of the biocatalyst on the surface of the electrode [105]. Indeed, electrochemical biosensors, whose principle is based on the use of an electrochemical transducer, are characterized above all by their adaptability, robustness, high sensitivity, wide detection range, easy miniaturization and fast response [88]. Due to the excellent structural and functional properties of Chit, it is used as a novel biocompatible material to produce electrodes and bind biocatalysts for advanced bio-fabrication materials.

Chit biopolymer maintains the biocatalytic functions which increase the stability by the covalent immobilization through the $–NH_2$ groups. A GOx based-biosensor was developed consisting of Pd@Pt core–shell NCs which coated with Chit throughout the covalent linking. The presence of Pd@Pt NCs facilitated and enhanced the direct electron transfer (DET) in the biosensor. The developed biosensor exhibited excellent performance with a sensitivity of 6.82 μA cm^{-2} mM^{-1} in the range of 1–6 mM for the detection of glucose substrate [106]. This design offer opportunities to develop electrochemical biosensors for detection of different analytes with low-cost and high sensitivity functions. An amperometric hemoglobin (Hb) biosensor for the detection of organic nitromethane (CH_3NO_2) was constructed [107]. The electrochemically conductive reduced graphene oxide (rGO) and the biopolymeric Chit matrix film were used to confine hemoglobin (Hb) on a GCE. This novel electrochemical Hb-CS/rGO-CS biosensor exhibits direct electrochemistry with a faster ET rate (6.48 s^{-1}) and excellent catalytic activity towards CH_3NO_2. The appropriate selection of the immobilization surface plays a crucial role in the development and advancement of biosensors. As explained in more detail in Section 5.2, layer-by-layer (LbL) assembly is another suitable technique for immobilizing enzymes while preserving their activity to improve the sensor's overall sensing capability [108]. Moreover, this technique offers to control the film architecture due to their high flexibility to develop new kind of films having various compositions with different functionalization units. In a similar fashion and using the LbL approach, biosensors have been developed by using different functionalized materials and employed as catechol biosensors (Fig. 9) [109].

Copper containing enzymes tyrosinase (Tyr) or laccase (Lac) working as catalyst and converting phenols to quinones were immobilized and deposited on alternating layers of a cation linker of CHI and anionic electrocatalytic materials of sulfonated copper phthalocyanine, CuPcS or AuNP, respectively (Fig. 9 (Left)). Electrochemical responses of the LbL platform towards catechol (Fig. 9 (Right) was studied and compared to the non LbL. The LbL platform revealed higher efficiency and

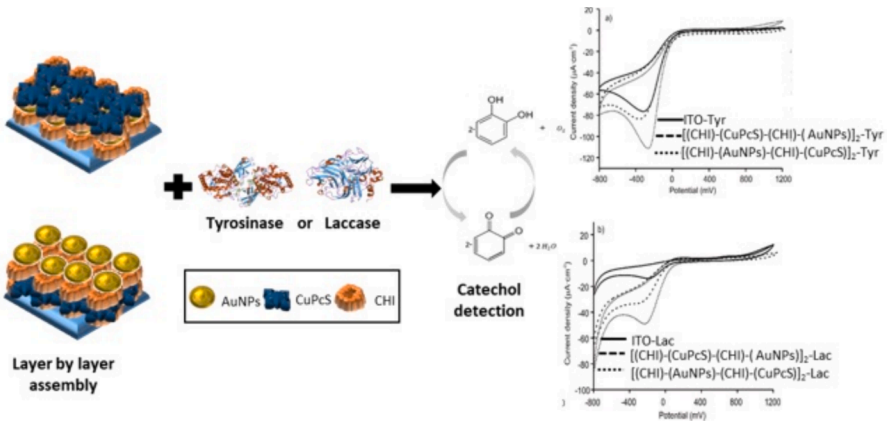

Fig. 9. (Left) The developed bisensing platform. (Right) Voltammetric curves of catechol 10^{-4} M in 0.01 M phosphate buffer pH 7 at an (a) ITO-Tyr (solid line), [(CHI)-(CuPcS)-(CHI)-(AuNPs)]2-Tyr (dashed line), [(CHI)-(AuNPs)-(CHI)-(CuPcS)]$_2$-Tyr (dotted line); and (b) ITO-Lac (solid line), [(CHI)-(CuPcS)-(CHI)-(AuNPs)]$_2$-Lac (dashed line), [(CHI)-(AuNPs)-(CHI)-(CuPcS)]$_2$-Lac (dotted line).Reprinted from [109] under the Creative Commons Attribution License.

improvement of the ET path between Lac and Tyr and the surface of the electrode, hence increases the intensity response. Furthermore, it shows lower LOD with a one order of magnitude for the [(CHI)-(AuNP)-(CHI)-(CuPcS)]$_2$-Tyr resulting higher surface roughness and bigger pore size, confirmed with AFM, which facilitated the diffusion of catechol. This study shows that the rational design of the electrochemical biosensors considering the functionalities and physicochemical properties of the materials is an excellent and effective strategy to improve and enhance the overall electrochemical performance of the biosensor.

5. Techniques of Chit deposition in (bio)sensors

Chromatographic techniques such as gas chromatography and high-performance liquid chromatography have been used to detect very low concentrations of analytes. However, these conventional techniques have disadvantages such as longer processing time, need for trained personnel, complex instrumentation, etc. Therefore, sensor-based devices have emerged as an alternative to these techniques as they offer advantages such as high sensitivity, rapid analysis, ease of use, and cost-effectiveness [110–112]. In this section, we will discuss different techniques of chit deposition in chemosensors and biosensors.

5.1. Molecular imprinting

Molecular imprinting (MI) is one of the techniques of Chit deposition in sensors. It works by synthesis of molecularly imprinted polymers (MIPs) in the presence of template molecule by polymerization of monomers (Fig. 10). The target molecules are eliminated after polymerization and resultant polymer can be utilized in sensors for selective extraction. The MIPs have received a lot of attention due to their easy synthesis, low cost, reusability and good chemical and mechanical stability. The MIPs have application not only in sensors but also in drug delivery, separation and environmental remediation [113,114].

Jiao et al. synthesized a sensor for identification of perfluorooctane sulfonate by doping Chit (CS) hydrogel with carbon dots [115]. The hydrogel was synthesized by polymerization reaction of epichlorohydrin with Chit to form new hydrogen bonds between amino groups and carbon dots. The results indicated that MIP hydrogel showed better performance as sensor than non-imprinted polymer. Shen et al. developed a new method to synthesize polymer for bacteria recognition by exploiting tendency of bacteria to self-assemble at oil–water interface [116]. In this method acryl-functionalized CS was treated with two different groups of bacteria and resultant was used for stabilising oil–-water emulsion made of monomers mixed in aqueous buffer. The oil phase polymerisation and bacteria removal result in a polymer bearing bacteria imprint. The bacterial recognition depends on type of pre-polymer and targeted bacteria. The synthesis of *N*-acryl Chit was done by reacting acryloyl chloride with Chit amino groups.

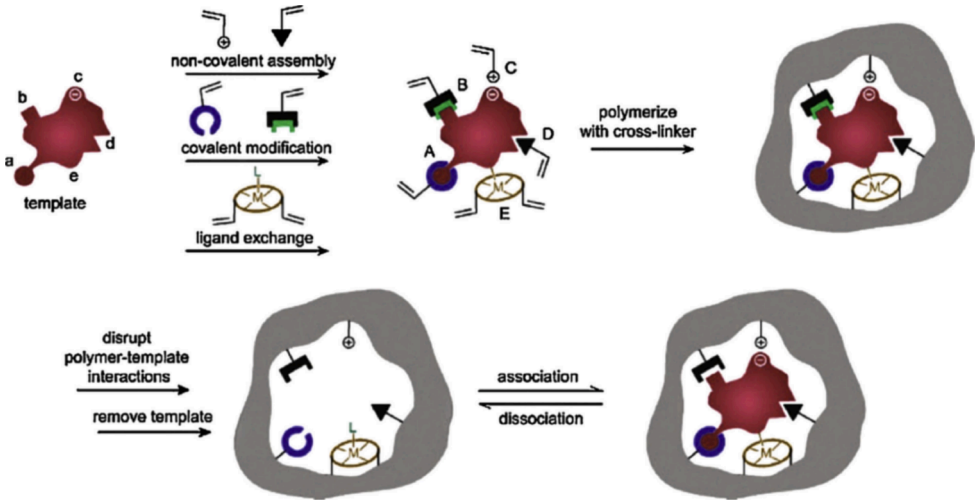

Fig. 10. Schematic representation of the MIPs preparation. Reprinted from [114], Copyright (2017), with permission from Elsevier.

5.2. Layer-by-layer deposition

One of the challenges in the search for novel biosensors with better stability and sensitivity is the effective immobilization of the elements to be detected. Techniques such as sol–gel entrapment, covalent bonding, adsorption and layer-by-layer (LbL) self-assembly can limit the movement of the detection element [117–120]. The LbL method is a simple and efficient method because the mild conditions minimize denaturation of the protein by adsorption of the polymer from an aqueous solution onto a solid support. It is cost-effective as only a very small amount of material is needed to create a thin layer. The only problem could be the slow electron transfer between the redox center of the enzyme and the electrode surface, which can be minimized by using nanomaterials such as graphene, carbon nanotubes, etc [121]. The use of graphene as one of the components offers the advantage of the outstanding electrochemical properties of graphene combined with the versatility of the LbL method, resulting in high sensitivity and an advanced biosensor.

Barsan et al. synthesized multilayer film by using nitrogen doped graphene (NG) dispersed in glucose oxidase (GOD) enzyme and Chit 1% (V/V) in acetic acid [122]. The positively charged Chit polymer (Chit+(NG + GOD)) and negatively charged polymer polystyrene sulphate were assembled using gold electrode substrate alternatively between the (Chit+(NG + GOD)) polymer and polystyrene sulphate polymer solutions. The specific capacitance values were calculated using CV data and it was found that nitrogen doped graphene containing Chit hawed change in specific capacitance from 59.6 μFcm^{-2} for pure gold quartz crystal to 341 μFcm^{-2} for first layer deposition then decreases to 245 μFcm^{-2} for second layer deposition. However, in the absence of glucose oxidase layer there is a slow decrease in capacitance with increase in Chit+(GOD) layer from 58.6 $\mu F.cm^{-2}$ for bare gold quartz crystal to 43.6 μFcm^{-2} and 40.4 μFcm^{-2}, for first layer deposition and second layer deposition, respectively. This capacitance variation is showing the importance of nitrogen doped graphene in multi-layer film. The CV results were validated from EIS study which showed high value of capacitance and small charge transfer resistance for nitrogen doped graphene containing multilayers. This biosensor formed from this multilayer film operates at potential ~ -0.2 V which is required to reduce the effect of interferents and has high sensitivity compared to other biosensors operating at ~ -0.2 V.

5.3. Cathodic electrochemical deposition

The cathodic electrochemical deposition (CED) of Chit is a mild, low cost and green method to synthesize composite or functionalized Chit materials because the local increase of pH due to the electrochemical cathodic half-reaction induces the chemical precipitation of Chit that englobe in its structure relevant species present in the electrolytic solution. The Chit film can grow according to a layer-by-layer assembly, as shown by Xu et al. [123]. They deposited a composite alginate/Chit film that show very good swelling performance and mechanical properties at dried states, as schematized in Fig. 11.

This method is preferred especially for the preparation of Chit/CNTs materials, as CNTs are excellent candidates for (bio) electrochemical sensors due to their high conductivity, chemical stability, and mechanical strength. The method of preparation of CNTs electrode was complicated because the involvement of organic solvents. Various strategies such as covalent modification of CNTs and non-covalent functionalization with surfactants and polymers have been used to make CNTs soluble in water [124]. Gorski et al. prepared a dehydrogenase biosensor by mixing CNTs in Chit solution [125]. The fabrication of electrode was done by casting CNTs solution on electrode surface, due to which sensor was accompanied with disadvantage of uncontrollable thickness of CNT-Chit films. Also, in this biosensor the immobilization was due to crosslinking with glutaraldehyde, which was not biocompatible for enzymes. So, there was need of some other modification in CNTs-Chit biosensors to make them compatible for enzyme recognition.

Luo et al. synthesized Chit-CNTs nanocomposites using simple and controllable method of cathodic electrochemical deposition [126]. This nanocomposite has good electrocatalytic ability for reduction and oxidation of hydrogen peroxide. Further, a biosensor based on CNT-Chit-enzyme nanocomposite was synthesized using simple one step electrodeposition method. In this method multiwalled carbon nanotubes and Chit were dissolved using ultrasonication. Polished and clean gold electrodes were connected to direct current power supply and dipped into the Chit-CNT solution (pH = 5). The H^+ ion from solution reduced to hydrogen at cathode. Thus, the pH at the cathode will start increasing once the pH of solution exceeds the pK_a of Chit (~6.3), Chit becomes insoluble and Chit entrapped CNTs starts to deposit at cathode surface. SEM analysis showed wire-like structure of diameters (40–80 nm). These were attributed to the Chit chains wrapped CNTs. The thickness of Chit-CNTs nanocomposite deposition can be controlled through the deposition time, concentration of Chit solution and applied voltage.

Juska et al. fabricated a dual enzyme electrochemical biosensor based on gold foam deposits and multiwalled carbon nanotubes (MWCNTs)-Chit nanocomposite [123]. The microfabricated gold band array electrodes were modified first by gold foam to increase the surface area. Then this deposited nanostructured gold foam served as 3D-matrix for MWCNT-Chit nanocomposite electrochemical deposition. The resulting electrode was highly conducting, biocompatible and suitable for attaching versatile chemical and biological agents. The glucose oxidase and horseradish peroxidase enzymes were successfully immobilized on MWCNT-Chit nanocomposite surface.

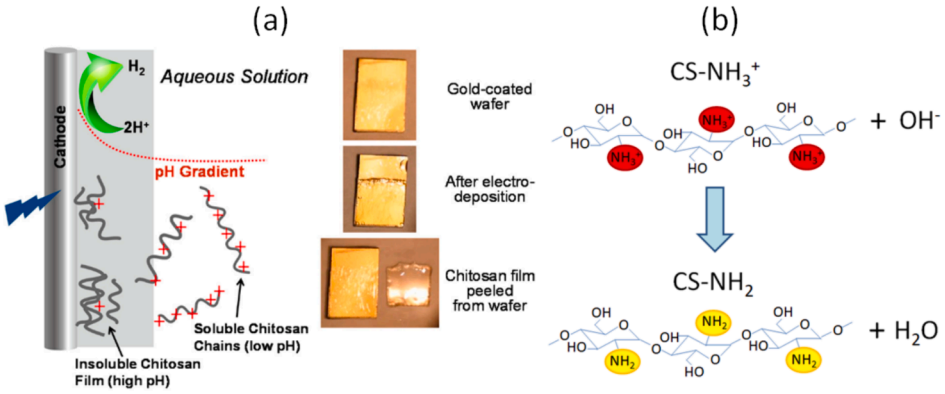

Fig. 11. (a) Illustration of the electrochemical deposition process used to fabricate a Chit electrode. Reprinted from [123] under the terms of the Creative Commons Attribution license. (b) Deposition of Chit (CS) in high pH solution.

5.4. Performance of Chit-based (bio)sensors deposited by MI, LbL, CED techniques

In the last years, a consistent number of studies have been conducted on (bio)sensors containing Chit as active material [64,127–130]. Thus, we have selected in this review some representative papers based on the methods of synthesis discussed in Sections 5.1-5.3.

5.4.1. Medical diagnosis

Oxygen for the human body has fundamental importance for the survival and proper functioning of the organism; however, the presence of incorrect amount of oxygen may indicate some problems. The concentration of oxygen present in the blood is closely linked to cellular apoptosis, tissue damage or cancer development [131] and can vary from 50 to 130 mm Hg while in cerebrospinal fluid varies from 1 to 10 mm Hg during carotid compression. In view of these data the possibility of using biomedical sensors able to detect the concentration of oxygen in the blood in a wide range of values represents a great step forward in the ease of examination, minimizing the critical time of diagnosis of pathological oxygen imbalance and reducing the delay of intervention on the patient [132,133].

Recently the oxygen sensors in the human body based on hemoglobin (Hb) are regaining huge interest in their great variety of application and have unique properties as hemoglobin exists in living systems and it is a natural oxygen carrier. However, the unfavorable orientation of hemoglobin makes it impossible to transfer electrons quickly to the electrode. Lots of efforts have been done to increase electrochemical kinetics, for example by adding nanoparticles to the electrode surface, bio membranes or polymer films. Alternatively, it is possible to use microcapsules containing high amounts of hemoglobin coated with Chit (Hb-CS). These microcapsules have demonstrated a great electrochemical sensitivity towards oxygen and are an excellent carrier for its transport [134]. $CaCO_3$ is used as a support for the insertion of hemoglobin, and used to deposit particles of hemoglobin, these subsequently begin to bind via cross-linking and then being completely coated with Chit to give rise to a microcapsule. The final microcapsule is obtained by melting the internal sphere of $CaCO_3$ through Na_2EDTA. Separately the glass carbon electrode (GCE) is coated by electrodeposition with Au-NP on which the Hb-CS microcapsules are deposited. The electrochemical characterization of the sensor has been performed in an electrolytic cell containing GCE/Au-NPs/Hb-CS as working electrode, a platinum wire as counter electrode and a calomel electrode (SCE) as reference electrode using voltammetric cycles in a phosphate buffer solution at pH = 7.4 and the results demonstrated a great sensitivity to oxygen in a wide range of molar concentrations from to 8.0×10^{-8} to 1.2×10^{-3} M [135].

Reactive oxygen species (ROS) such as peroxides, superoxides and hydroxyl radicals are natural by-products of oxygen metabolism and play an important role in cell communication [136]. Hydrogen peroxide can regulate the metabolism of a cell in a living system. In signal transduction, low concentrated H_2O_2 works as second messenger while high H_2O_2 concentration cause cell oxidative stress and physiological disorders [137]. Knowledge of hydrogen peroxide concentration is therefore of fundamental importance for physiology and pathophysiology, but its detection in the body is rather difficult due to the lack of efficient probes because of the small size of cells and the short half-life of intracellular superoxide radicals [138].

The use of special construction techniques allowed to overcome these disadvantages and guaranteed the possibility of creating an efficient and functional H_2O_2 sensor. Catalase is an enzyme, belonging to the class of oxydoreduttase, involved in the detoxification of the cell from reactive oxygen species. Catalase were immobilized on Chit (Chit-CAT), and a ferrocene β-cyclodextrin complex (β-CD-FE) is also prepared to improve sensor sensitivity because electron transfers from electrode easily towards the enzyme and act as a mediator [139]. At low oxidation potential electronic mediator distributed in the CS membrane react fast and reduces enzyme. The sensor consists of the composite Chit-CAT/β-CD-FE on the electrode surface (Fig. 12) immersed in a saline phosphate buffer solution (PBS) at 7.0 pH. The Chit-CAT coated electrode A and the Chit-CAT/β-CD-FE composite electrode delivered a high current signal peak in response to H_2O_2 representing a high sensitivity to hydrogen peroxide. This can be explained by the fact that catalase was oxidized by hydrogen peroxide which later, after obtaining electrons from the electrode, reduces. Catalase oxidation significantly increases the current peak. Chit-CAT/β-CD-FE composite electrodes are more sensitive to the presence of H_2O_2 than the A electrode because β-CD-FE (the electronic mediator) helps to increase the electrical signal strength of Chit-CAT/β-CD-FE composite electrode than A electrode. In this work, it is also shown how the pH value of the solution affects the current peak of the sensor. As the pH increases, the current increases to an optimum value up to pH = 7 and then decreases again. The chronoamperometric analysis shows that the composite electrode responds quickly to the presence of H_2O_2 and the reaction current rapidly increases and saturates within 5 s. It suggests that this electrode have good catalytic behaviour. Also, the current peak increases linearly with H_2O_2 concentration (Fig. 12b). The sensor was also stored in saline phosphate buffer in a 4 °C chiller for 10 days, recorded a decrease in response of only 9.6% indicating that the composite electrodes are stable.

5.4.2. Environmental monitoring

Environmental monitoring involves the measurement, evaluation and determination of environmental parameters or pollution levels, whether periodically or continuously, with the aim of preventing adverse and harmful effects on the environment. Environmental monitoring therefore consists of a complex investigation process that combines the observation and measurement of certain bio-parameters over time. The areas of application range from the protection of human health to the control of industrial processes and for safety purposes. Sensors can be used for the detection of pesticides, pollutants, highly flammable or hazardous substances and toxic substances in the air. The development of new sensor technologies for environmental control helps to simplify or detail the type of research to be carried out; it also improves the reliability or cost of current technology.

Organophosphate (OP), a common pesticide for pest control, is highly toxic for environment and has lots of side effects. It is harmful to human life because it releases the residues in stuffs in which it is used as pesticide, e.g. food and water. Rapid determination and minimum quantification of OPs is the solution to save human health and protect environment. Liquid or gas chromatography coupled with mass spectrometer have been widely used as traditional analytical methods to determine the trace of OPs. These methods are not very suitable because it need huge manpower as well as resources too. For on-site surveys these methods are not feasible because it requires intricate pretreatment. In recent years, enzyme biosensors have been developed as an alternative method for pesticides detection and arise as a promising solution. Among these, inhibition biosensor systems, based on the immobilization of acetylcholinesterases (Ache) on various electrochemical transducers, have shown satisfactory results for the analysis of pesticides in which enzyme activity has been used as an indicator of quantitative insecticide measurements [140].

A major challenge for the manufacture of biosensors is the effective immobilization of the enzyme on the electrode surface. The use of Chit is beneficial toward this result, due to its film forming, chemical and electrochemical properties. As discussed above, Chit can be deposited on an electrode through electrochemical technique. The solubility of the Chit depends on pH and also the electrodeposition triggers a local increase in pH which reduces the solubility of the Chit and $CaCO_3$ precipitates. Both compounds can be co-electrodeposited on the surface of a suitable substrate to form a 3D porous nano $CaCO_3$-CS structure (Fig. 13).

The electrodeposition process causes the formation of hydrogen bubbles on the surface of the electrode leading to the generation of a porous structure in the nano $CaCO_3$-Chit matrix [141]. This structure

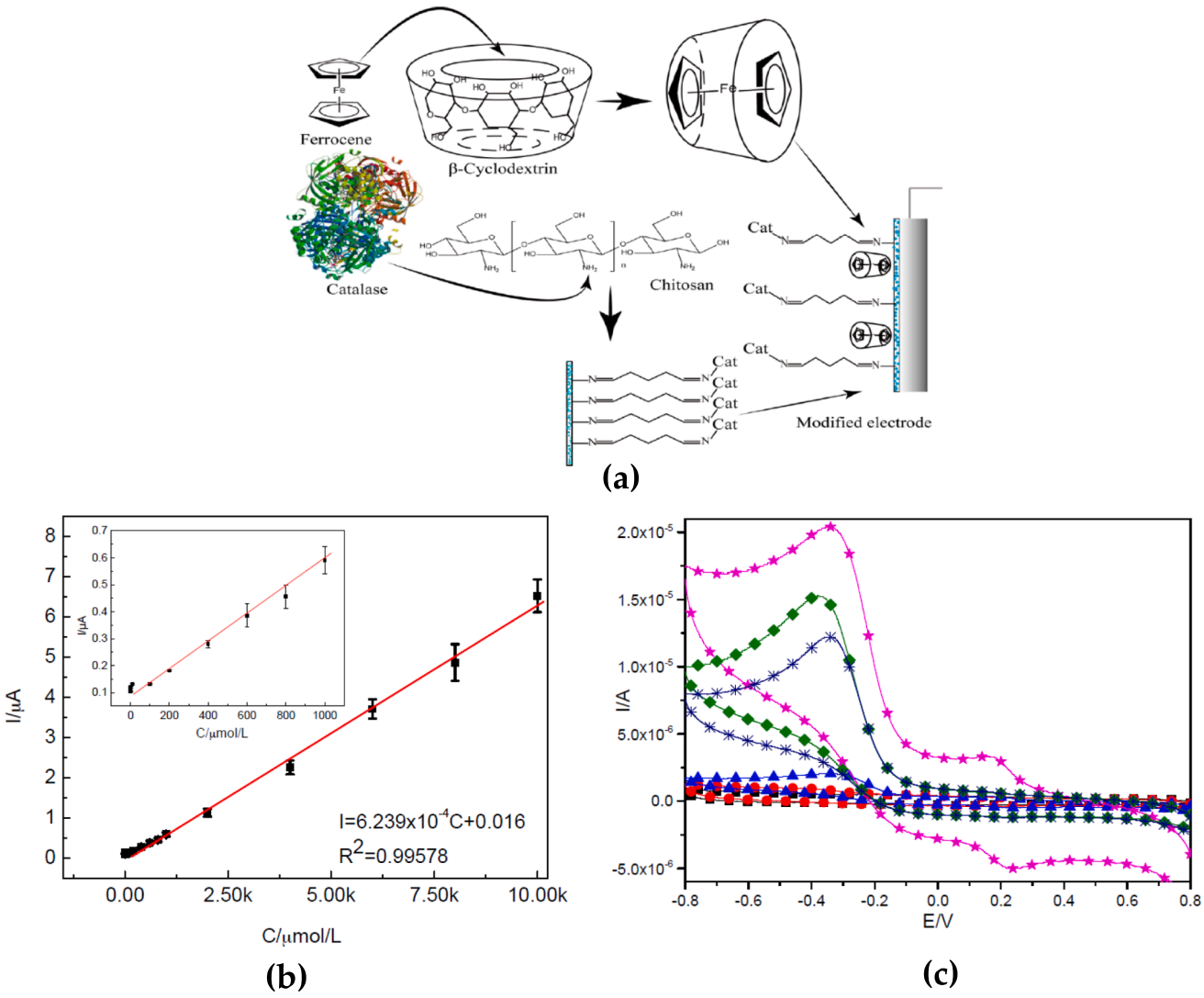

Fig. 12. (a) Scheme reporting the composite electrode Chit-CAT/β-CD-FE. (b) The reduction peak current as a function of H_2O_2 concentration. (c) voltammetric cycles of the▲ control electrode (■ bare electrode, ◆electrode A coated with Chit-CAT, ● electrode B coated with Chit) and ★the composite electrode Chit-CAT/β-CD-FE in an H_2O_2 solution. The bare electrode and electrode B showed no evident reduction activity in 0.1 mol/L PBS (pH 7) in the range of [-0.80 V- 0.80 V]. with permission under the terms of the Creative Commons Attribution license.
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guarantees a good permeability that favors the diffusion of the enzyme, the electronic transfer process, and the immobilization of Ache for the detection of OPs. The developed porous film is found to be stable and shows rapid detection of Parathion-Methyl, a particular type of organophosphate. The sensor was designed by electrodeposition of a glassy carbon electrode (GCE) in $CaCl_2$- NH_4HCO_3- Chit based electrolyte at constant potential of −3.0 V vs SCE. After the electrodeposition of the film the composite electrode was coated with Ache solution to obtain Ache-$CaCO_3$-Chit/GCE material. For the measurement of Paration-Methyl, the prepared electrode is immersed for 10 min in a saline phosphate buffer solution containing different concentrations of Paration-Methyl, then placed in the electrochemical cell at pH 7 saline phosphate buffer containing acetylcholine chloride (AchCl) and subjected to voltametric cycles to study its electrical response.

The voltametric analysis of the Ache-nano $CaCO_3$-Chit/GCE gave an oxidation peak at 0.79 V, however no noteworthy peak was noticed for nano $CaCO_3$-Chit/GCE. For Ache-nano$CaCO_3$-Chit/GCE, peak appears while thiocholine oxydation and Atcl produces during hydrolysis, catalyzed by the immobilization of Ache. Ache-nano$CaCO_3$-Chit/GCE has shown greatly improved electrochemical behavior than Atcl and Ache/GCE, with the oxidation overvoltage negatively shifted of 90 mV. The presence of the porous layer plays an important role, facilitating diffusion, allows the oxidation of thiocholine possible at a lower potential and ensuring a platform for good achievement towards immobilization of the enzyme (Ache). The activity of the immobilized enzyme is the effect which appears in form of produced current and can be utilized as quantitative measure of OPs.

Paration-Methyl is one of the pesticides. In the Paration-Methyl solution, the current produced was drastically reduced exhibiting highly toxic nature, is engaged in the irreversible action of inhibition of Ache, therefore pesticide substrate could not perform high enzymatic activity. The presence of paration-Methyl can be easily detected in the significant changes observed in the voltametric response of Ache-nano$CaCO_3$-Chit/GCE. Increased amount of paration-methyl, decreases the amount of current produced by Atcl on Ache-nano$CaCO_3$-Chit/GCE.The detection limit is 1 ng/ml. No decrease was recorded in the peak AchCl current due to the presence of nitrophenol and nitrobenzene (electroactive nitrophenyl-derivatives) or any other oxygen containing inorganic ions.

The electrodeposition method is also used to obtain conducting Chit polymers composites (Fig. 14). Sadrolhosseini et al. [142] reported a

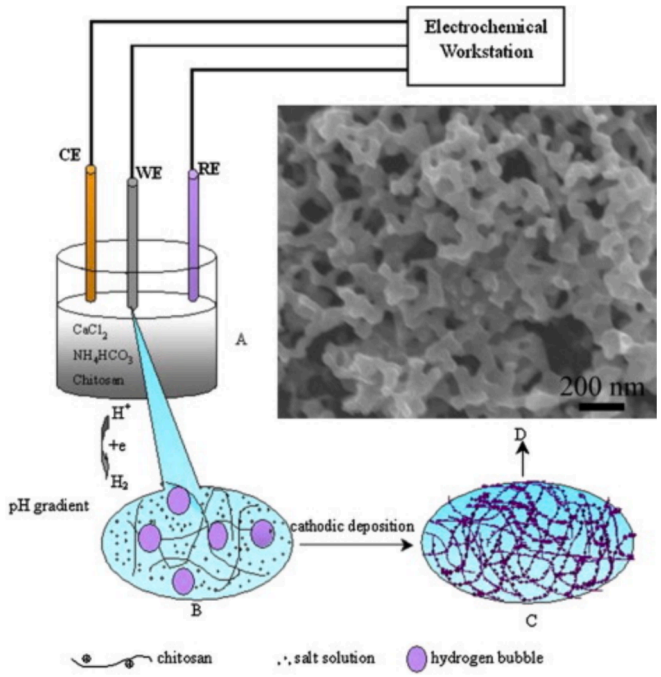

Fig. 13. Scheme of the co-electrodeposition process and porous structure of nano $CaCO_3$-Chit. Reproduced by [141] with permission from Elsevier. Reprinted from [141], Copyright (2009), with permission from Elsevier.

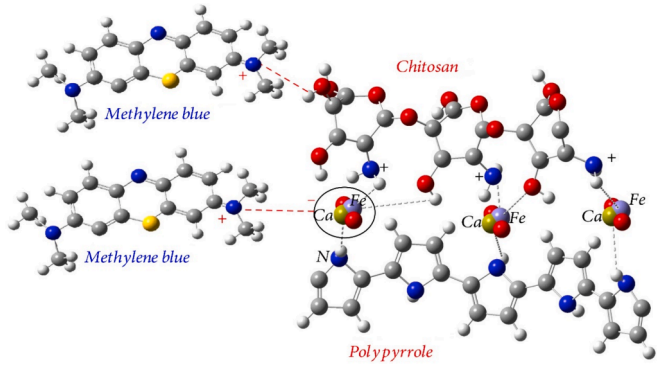

Fig. 14. Scheme of the chemical interaction between PPy-Chit-$CaFe_2O_4$ and methylene blue [142]. Reproduced under the Creative Commons Attribution License. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

polypyrrole-Chit-calcium ferrite nanocomposite which was electrodeposited on a gold coated electrodes from few nanometers up to about 60 nm to detect and remove anionic and cationic dyes. A sensibility is achieved about ~ 0.01 ppm.

Layer-by-layer (LBL) method is employed to combine materials with different functionalities to obtain optimized electrochemical sensors. It allows a better interaction between particles and favours the electrochemical detection due to the increase of the sensing centers. It also offers higher roughness and porosity which enhance adsorption and provides good transmission rate to charges. Comino et al. [109] fabricated a Chit-based biosensor using LBL method. Chit was chemically modified using gold nanoparticles and sulfonic copper phthalocyanine. It offers the immobilization of phenol oxidases enzymes tyrosinase and laccase. This fabricated biosensor shows a two order lower magnitude of limitation in detection than other nanostructured devices.

Chit has primary amino groups which help in dissolving noble metals in its cationic and anionic forms and therefore metallic nanoparticles get easily stabilize in Chit solution depending on its pH. Thus, the properties of Chit allow it to be used as a metal/polymer matrix for the reduction of metal ion precursors in a polymeric ion exchange. New techniques have also been tested which have allowed the manufacture of modified Chit to obtain optical properties suitable for optical sensors with planar waveguides simply by changing its refraction index in situ by Chit motion. The operating concept of these sensors is based on power variations transmitted through waveguides, when the evanescent wave interacts with the absorbent medium [143].

Hydrogen sulfide is a toxic gas, largely produced by drilling and refining of oil or gas, waste water treatment, firing furnaces, tanneries or landfills [144]. In addition, H_2S is involved in a wide range of physiological functions and abnormal levels associated with a number of diseases, including diabetes, hypertension, stroke and Alzheimer's disease [145].

A special type of sensor capable of detecting hydrogen sulfide was tested with Chit modified with silver nanoparticles. After the preparation of the Chit film, film is immersed in a solution of $AgNO_3$ or H$[AuCl_4]$ at a concentration of $5{\cdot}0 \times 10^{-3}$ M for 10 min. After absorption of metal ion precursors, the substrates are immersed in a 0.01% solution of $NaBH_4$ for 30 min. For developing more effective sensors based on optical waveguide there is need to optimize minimum amount of sensitive material with respect to minimum thickness of the sensor so that evanescent waves should not decay exponentially. Generally, for effective sensing there is requirement of minimum mass loading of nanoparticles with at least 100 to 150 nm layers of polymer material. The influence of the concentration of precursors in solution and the absorption time before metal reduction with $NaBH_4$ have been characterized by measuring absorption limit which should be maximum of 10 min and the concentration of $AgNO_3$ should not exceed $5{\cdot}0 \times 10^{-3}$M. Chit composite with gold and silver nanoparticles was achieved by preabsorption of H$[AuCl_4]$ and $AgNO_3$ and further $NaBH_4$ was reduced. TEM study confirmed uniform distribution of all the materials in the composite. The average size of gold and silver particles was reported around 10 nm. The homogenous coating of silver and gold nanoparticles was found to be optically transparent. The plasmonic absorption peak was recorded at 410 nm and 530 nm for silver and gold respectively. The stability of these metallic nanoparticles was checked in polymer matrix after storing them for long time. In ambient conditions its optical properties were not deteriorated even after two months.

Another special type of sensor based on Chit and capable to detect dibutyl phthalate (DBP) was recently prepared [146]. In this system F-CC3 film was formed by casting functional corncob biochar (F-CC3) on glassy carbon electrode (GCE).

Then, the pretreated GCEs were further modified by means of molecularly imprinted polymers synthesized with Chit as functional monomer, DBP as the template molecule and glutaraldehyde as the crosslinking agent. The sensor was characterized in terms of cyclic voltammetry, differential pulse voltammetry and electrochemical impedance spectroscopy. This sensor exploits the molecularly imprinted technique and F-CC3 biomass materials to achieve high selectivity and high sensitivity for the determination of DBP. The results reported in this work show that the sensor presents a good linear relationship between the response current and the DBP concentration ranging of 0 ~ 1.8 μM with a limit of detection of 0.0026 μM. Moreover, this sensor was proven to be useful in detection of DBP in rice wine purchased from market.

To detect the presence of cadmium in daily use water and milk products, a delicate electrochemical sensor has been successfully fabricated where imprinted electrodes are developed based on Chit/AuNPs/graphene altered glassy carbon [147]. The device could detect Cd(II) ions with 1.62×10^{-4}μM detection limit. The fabricated sensor found to be stable and highly sensitive. The detection of CD(II) ions was repeated, and measurements were found to be repeatable. In DPV response, the peak current ratio (I_s/I_0) was used to determine the selectivity of the device with different kind of metal ions eg. Pb(II), Cu(II), Co(II), Mn(II), Ni(II), Zn (II) and Fe(II). The DPV response of Cd(II) (0.5 μm) was

performed in 0.1 M acetic acid-sodium acetate solution (pH 5) using three electrode system. The measurements were repeated 10 times the check the repeatability of the fabricated device. To verify real applications thereof, the measurements were done to sense cadmium in daily drinkable products such as pure milk, tap water, and river water showing acceptable average recoveries. Overall, the results reported in this study highlight that the present device is very useful to determine the amount of Cd(II) ions in daily used products.

6. Conclusions

This review discusses recent research progress on Chit-based sensors and biosensors focusing the attention on (*i*) material science aspects of Chit functionalization, (*ii*) methods of synthesis, (*iii*) most relevant applications. The successful strategies for Chit modification with functional nanomaterials and their integration in Chit-based electrochemical (bio)sensors are highlighted.

The use of Chit in electrochemical biosensors offers several advantages. One key benefit is its ability to form stable films on sensor surfaces, providing an excellent platform for attaching biomolecules such as enzymes or antibodies. For example, in glucose biosensors, Chit films can effectively immobilize glucose oxidase, enhancing sensor stability and sensitivity. Additionally, Chit's biocompatibility makes it well-suited for applications in biological environments. In DNA biosensors, Chit-based matrices can facilitate the immobilization of DNA probes, allowing for precise detection of target sequences. Moreover, Chit's versatility extends to its electrochemical properties, where it can act as a mediator for electron transfer, enhancing the performance of electrochemical biosensors. Overall, Chit's ability to create stable interfaces and its compatibility with biological systems make it a valuable material for improving the sensitivity and reliability of electrochemical biosensing devices. Combining Chit with functional nanomaterials in electrochemical biosensors harnesses Chit's film-forming capability and biocompatibility alongside the unique properties of nanomaterials, resulting in enhanced sensor performance. For instance, coupling Chit with noble metal nanoparticles like gold or silver facilitates stable immobilization of biomolecules on sensor surfaces, improving the sensor's sensitivity and accuracy. In glucose biosensors, this synergy enables precise glucose detection due to the increased conductivity and catalytic activity of the Chit-nanomaterial composite. However, the fabrication process for these hybrid materials can be complex and resource-intensive, requiring specialized equipments and expertises. Additionally, while Chit-functional nanomaterial composites offer improved sensor performance, they may also exhibit increased susceptibility to fouling and nonspecific binding, leading to interference in biosensor measurements. Despite these challenges, the integration of Chit with functional nanomaterials represents a promising avenue for developing advanced electrochemical biosensors with enhanced sensitivity and reliability.

Future directions would involve: (*1*) the development of new strategies to avoid non-specific binding and reactions and reduce toxic materials; (*2*) due to the advances in functional and emerging (nano) materials, a broadening of modification and functionalization methodologies of Chit to meet the requirements and overcome challenges; (*3*) the improvement of stability under ambient and/or physiological conditions for longer periods of time; (*4*) toxicity studies side by side with the advancement of the new functional (bio)sensors; (*5*) a better focus on the upscaling procedures to minimize the production costs of portable electrochemical (bio)sensors; (*6*) the increase in biocompatibility and environmental assessment studies.

Among the techniques of Chit deposition in (bio)sensors, electrodeposition is the less known but the more promising because the intrinsic capability of structures patterning at the nano-and micro-scale, easy charge-controlled incorporation of carbon nanotubes or other nanomaterials and possibility of uniform co-deposition with other polymers. While other techniques (as LBL, lithography, MIP) are commonly employed for many sensing polymers, electrodeposition can be used for Chit in unique way by exploiting its pH-dependent solubility allowing the polymer to be directly assembled on the substrate and functionalized prior to its use. The electrochemical control at the nanoscale could notably improve signal-to-noise ratio that are fundamental for the intent of the analysis.

Finally, the applications already investigated and discussed in this review open the way to the study of novel use of Chit in (bio)sensors related to its unique green, biocompatible, biodegradable, chemical, film-forming features.

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CRediT authorship contribution statement

Patrizia Bocchetta: Conceptualization, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing. **Ali Othman:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing. **Meenal Gupta:** Writing – review & editing. **Giovanni Andriani:** Writing – original draft. **Patrick Martin:** Writing – original draft, Writing – review & editing. **Yogesh Kumar:** Writing – original draft. **Nicolas Joly:** Writing – original draft. **Pasquale Sacco:** Writing – original draft, Writing – review & editing. **Muhammad Sufyan Javed:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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