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Recent advances in chitosan-based electrochemical sensors and biosensors

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Abstract

Chitosan is a biopolymer derived from chitin. It is a non-toxic, biocompatible, bioactive, and biodegradable polymer. Due to its properties, chitosan attracted considerable attention in several fields such as agriculture, food industry, medicine, paper fabrication, textile industry, and water treatment. In addition to these properties, chitosan has a good film-forming ability which allows it to be widely used for the development of sensors and biosensors. This review will be focused on the use of chitosan-based composites for the preparation of the electrochemical sensors. It also aims to provide an overview of the advantages of using chitosan as an immobilization platform for biomolecules by highlighting its applications in electrochemical biosensors. Furthermore, the application of molecularly imprinted chitosan for the preparation of the electrochemical sensors will be discussed.

Keywords: Chitosan, composite, immobilization of biomolecules, molecularly imprinted polymer**,** electrochemical sensor; biosensor.

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1. Introduction

Chitosan is a biopolymer derived of chitin which is a structural polymer of the exoskeletons of all arthropods (crustaceans and insects) and endoskeletons of cephalopods (cuttlefish, squid, ...). It is obtained mainly from the shell of shrimp. The deacetylation reaction of the chitin in a basic medium allows producing chitosan. This later is a linear copolymer composed by repeat units of D-glucosamine and N-acetyl-D-glucosamine, linked by a ß-bond (1→4) [1]. Chitosan has attracted considerable attention because of its many potential applications in agriculture [2], food industry [3], medicine [4], textile industry [5], and water treatment [6].

Chitosan is widely used for the preparation of sensors and biosensors thanks to its non-toxic, biocompatible, and biodegradable properties, and especially its film-forming ability [7,8]. A sensor is a device that translates biological, physical, or chemical information into a quantifiable signal (electrical, optical, thermal ...). The electrochemical sensors are highly sensitive and selective towards various types of analytes [9]. They allow a faster and easier analysis than conventional analytical techniques such as chromatography, mass spectrometry (MS), and nuclear magnetic resonance (NMR). The macromolecular chain of chitosan can be easily modified with nanoparticles and conductive polymers to produce composites with high electron transfer rate and specific surface area for the development of the electrochemical sensors [10,11]. Also, thanks to the free amine and alcohol groups, chitosan is largely used for the immobilization of biomolecules such as enzymes [12], DNAs [13], and antibodies/antigens [14] for the preparation of electrochemical biosensors.

Molecularly imprinted polymer (MIP) is a material obtained by the polymerization reaction of a functional monomer in the presence of a template. The molecular imprinting allows the formation of the specific recognition sites (cavities) in polymer matrices. For this reason, these materials have an affinity for the original molecule. The MIP rebinds to the target molecule by the same mechanism as that between the antibody and antigen [15]. Chitosan is an outstanding candidate for the preparation of MIPs thinks to its amine and alcohol groups which are capable to react with various types of cross-linkers and to create the specific cavities for the several types of analytes. Molecularly imprinted chitosans (MICs) are widely applied for developing robust electrochemical sensors for industry, diagnostics, and environmental analysis [7].

In this review, we describe the various properties of chitosan and its application for the development of the electrochemical sensors and biosensors focusing on the last 5 years. This manuscript begins with some generalities of chitosan, then the chitosan-based composites for the preparation of the electrochemical sensors, and ends with the application of chitosan as an immobilization platform of biomolecules for the development of the electrochemical biosensors. The use of the molecularly

imprinted chitosan for the preparation of the electrochemical sensors will be also discussed.

2. Preparation, properties, applications, and chemical modifications of chitosan

2.1. Chitosan preparation

As mentioned above, chitosan is a natural cationic polysaccharide (in dilute acid medium) derived from chitin. The deacetylation reaction of chitin in a basic medium leads to chitosan (figure 1). The chemical structure of chitosan is a chain composed of two monomeric units: deacetylated D-glucosamine and N-acetyl-D-glucosamine linked via a glycosidic bond (1→4). The chitin and chitosan structures differ only in the groups linked to carbon 2 which are acetamide for chitin and amine for chitosan [16].

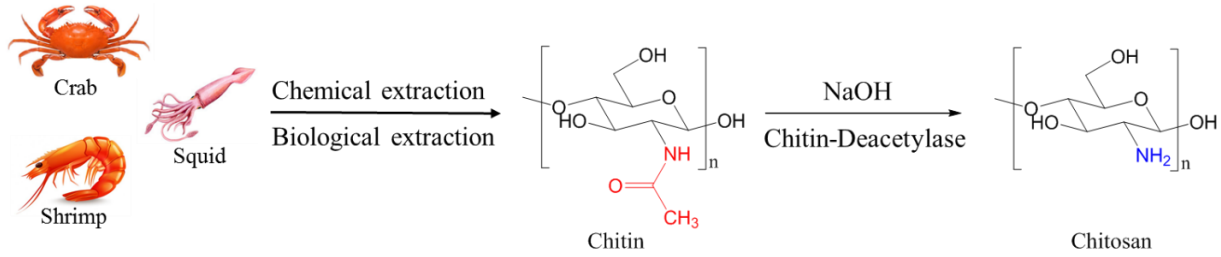


Figure 1: Preparation of chitin and chitosan

The distinction between chitin and chitosan is based on the degree of deacetylation (DDA), which is the proportion of amine groups to acetyl groups that are present in the polymer chains. Chitosan has a DDA that varies between 50 and 99% with an average of 80%; it is highly dependent on crustacean species and preparation methods. However, chitin has a DDA below 50%. Indeed, at a DDA higher than 50%, chitosan becomes soluble in diluted acidic aqueous solutions. Moreover, it has free amine groups which are active sites in chemical reactions.

2.2.Properties of chitosan

The properties of chitosan depend on DDA and molar mass; these parameters influence the physical, chemical, and also biological properties. Crystallinity is also an important parameter because it controls many properties such as swelling in water. Chitosan is generally a semicrystalline material, it crystallizes in the orthorhombic system and two types of products are available: chitosan I with a low DDA is more disordered than chitosan II with a high DDA [17].

The presence of the amine functions, the charge, and the properties of chitosan vary with the pH. At low pH (3-4), the amines protonate and become positively charged. It is the only polycation from natural origin. Whereas at high pH, chitosan cannot attract hydrogen, so it does not gain positive charges and will remain insoluble [18].

Chitosan shows better adhesion properties to negatively charged surfaces [17]. Its cationic character in an acid medium allows it to react with negatively charged biological compounds. Besides, the nature of the glycosidic bonds also gives chitosan an excellent film-forming property.

Chitosan presents several biologic properties, it is non-toxic, biocompatible, bioactive, and biodegradable polymer [19]. Also, its antimicrobial activity has been extensively described and published in recent years for its action against a wide variety of microorganisms including bacterial and fungal species. Chitosan inhibits the growth of many parasites and reduces the development of infections [4].

2.3.General applications of chitosan

Chitosan has many applications in different fields because of its various properties listed above especially its polycationic character which is unique among natural polymers. It finds applications in economically promising sectors such as the food [3], cosmetics [20], pharmaceutical [21], agricultural [2] industries, and in the development of sensors and biosensors [22,23]. Indeed, several of research works have made possible to study and develop the use of chitosan as an encapsulation material for drugs. This would allow the controlled release of the drug or any other substance [24]. Chitosan is also largely used for water treatment thanks to its chelating property which allows it to eliminate heavy metals, even in very small quantities [25]. Moreover, in the field of cosmetics, the film-forming and cationic properties of chitosan are exploited in many hair and skincare creams and lotions [26]. Also, in agriculture, chitosan is capable of triggering defense mechanisms in plants against infections and parasitic attacks [27].

2.4.Chemical modifications of chitosan

The presence of reactive functional groups as well as the polysaccharide nature of chitosan, allows it to perform numerous chemical modifications (figure 2). Amine and hydroxyls groups can provoke chemical reactions such as alkylation, reaction with aldehydes, epoxides, and ketones, etc [28–30]. These reactive groups allow chitosan to be easily transformed into gels, films, nanofibers, and nanoparticles [31–33].

3. Electrochemical sensors based on chitosan

An electrochemical sensor is a device that allows the translation of biological, chemical, or physical information into an electrical signal. The electrochemical sensors are widely used in chemical analysis and research in many fields such as the chemical industry, food industry, environment, pharmacy, etc [34].

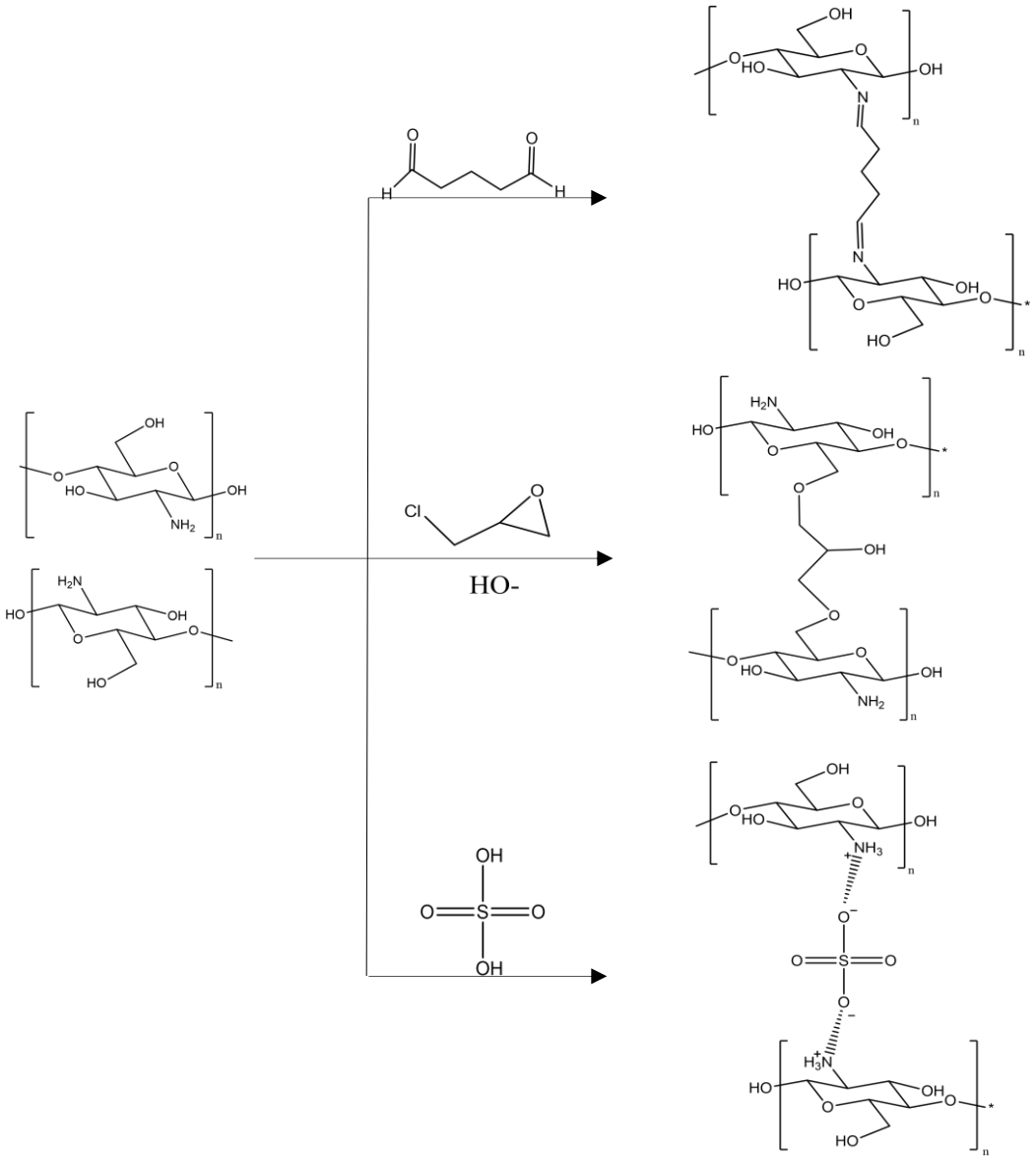
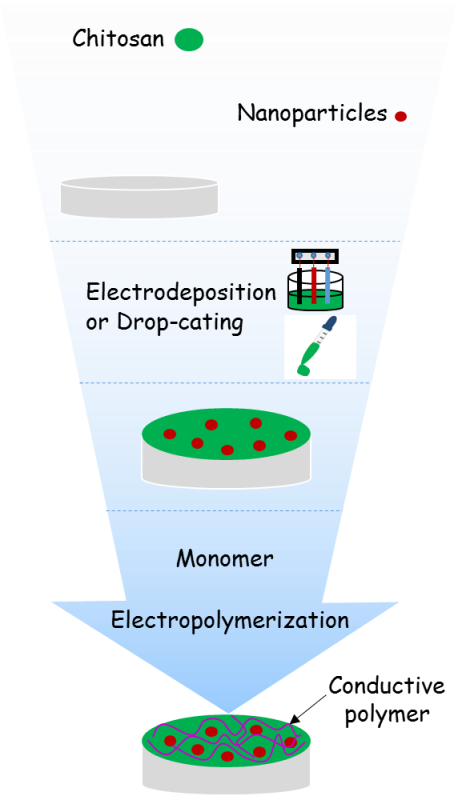


Figure 2: Chemical modifications of chitosan

Chitosan is a functional material that shows good adhesion, film-forming ability, and biocompatibility properties [35], which are favorable for the fabrication of the sensors. Chitosan does not exhibit electrical conductivity, therefore it is generally combined with nanoparticles like graphene [36], and multiwall carbon nanotubes [37] as well as with conducting polymers such as polypyrrole [38] and polyaniline [39] to enhance its electrical properties for sensing applications (Scheme 1).

3.1.Electrochemical sensors based on chitosan and nanomaterials

The polymeric chain of chitosan can be easily modified with various types of nanoparticles, which makes it suitable for the development of nanocomposites films. Thanks to the large specific surface area and very high charge transfer of the nanoparticles, chitosan nanocomposites are widely used in sensors construction (Table 1).



Scheme 1: Preparation process of the electrochemical sensors based on chitosan composites

The development of chitosan nanocomposites based sensing platforms have been considered as an attractive topic due to their mechanical, physicochemical, and electrical properties. Besides, they provide high sensitivity to detect low levels of analyte. Indeed, Lou et al. [40] developed an electrochemical sensor for the amlodipine identification, using a porous nickel molybdate nanosheets-chitosan nanocomposite modified glassy carbon electrode. This device shows an improved electrochemical performance of the sensor compared to some of the electrochemical sensors developed for the determination of amlodipine such as the sensors prepared by multi-walled carbon nanotubes and graphite modified paste electrode [41] or multi-walled carbon nanotubes modified the gold electrode [42]. A similar case was observed with a sensor based on chitosan- zeolitic imidazolate framework-8 (ZIF-8)-acetylene black nanocomposites for rutin determination [43]. The chitosan was used as a dispersive agent of ZIF-8 and acetylene black. Then the obtained dispersion was dropped onto the surface of glassy carbon electrode and dried under an infrared lamp. The authors reported that the sensor has high sensitivity, good stability, and reproducibility towards rutin.

Chitosan-multiwall carbon nanotubes (Chitosan-MWCNTs) nanocomposites have gained interest as an attractive material to increase the sensitivity of electrochemical sensors by increasing the electrode surface area and improving the electron transfer. Generally, the positive charge of amino groups of chitosan is self-assembled via electrostatic interactions with the negative charge of carboxylic groups of

the functionalized MWCNTs. For example, Akhter and colleagues fabricated an electrochemical sensor based on MWCNTs-chitosan-Co nanocomposite for the detection of the paracetamol [44]. The MWCNTs have been electrostatically attached in the chitosan chain, then the obtained nanocomposite was used to immobilize cobalt ion. The fabricated sensor was successfully used for the determination of paracetamol in commercial human serum and tablets samples. Velmurugan et al. [45] published another research work for the determination of nitrofurantoin using MWCNT-chitosan nanocomposite incorporated nano-hydroxyapatite.

Gold nanoparticles are ideal candidates for the modification of chitosan biopolymer to prepare electrochemical sensors due to their high conductivity, absorbability, and catalyzing electrochemical reactions. In fact, Diouf et al. [22] developed a new electrochemical sensor by self-assembling chitosan capped with gold nanoparticles on a screen-printed carbon electrode. This sensor presents outstanding advantages for the determination of aspirin compared to other sensors such as those prepared by using graphene modified glassy carbon electrode [46] or ZnO nanoparticle ionic liquid composite modified carbon paste electrode [47]. In fact, this sensor exhibits good sensitivity and selectivity toward aspirin in human physiological fluids and tablets samples. Trani *et al*. [48] fabricated a selective electrochemical sensor for the detection of caffeine using chitosan-gold nanocomposite as a platform modifying the electrode surface. The sensor was then effectively used for the determination of caffeine in commercial beverages.

The electrochemical sensors based on chitosan-iron oxide (Chitosan-Fe_3O_4) nanocomposite have been used for the determination of a wide range of molecules, with a recent application for gallic acid in green tea [49]. The combination of chitosan with Fe_3O_4 nanoparticles enhances the oxidation current of gallic acid, reaching a LOD of 12.1 nM. The proposed sensor was effectively used for the determination of the gallic acid in green tea. Another example reported a sensor for the detection of bendiocarb based on Chitosan-Fe3O4 nanocomposite as a glassy carbon electrode surface modifier [50]. The electrochemical sensor showed great potential for bendiocarb identification.

Due to their unique electrical properties, large surface area, and good film-forming ability, chitosan-graphene nanocomposite has been largely used for the development of electrochemical sensors. In fact, Feng *et al.* [51] developed an electrochemical sensor based on chitosan/graphene oxide nanocomposite for the detection of melamine. The graphene oxide was then homogeneously dispersed in the chitosan solution due to the electrostatic attraction between them. The obtained sensor exhibits excellent sensitivity to melamine and was effectively used for its determination in milk samples.

Chitosan nanocomposites are also compatible with screen-printed electrodes (SPE). Indeed, Reddy et. [52] developed an electrochemical sensor based on conducting film of chitosan-gold nanocomposite

modified SPE surface. A square wave voltammetry analysis technique was used to detect ciprofloxacin with a micromolar detection limit of 0.001 µM. The author reported that the sensor was effectively employed for ciprofloxacin determination in biological samples.

Table 1: Typical examples of electrochemical sensors based on chitosan and nanomaterials**.**

Electrode	Analyte	Technique	Detection range (mol/L)	LOD (mol/L)	Real sample	Ref
HANPs/MWCNT-CS/GCE	Nitrofurantoin	AM	$0.005x10^{-6}$ - $982.1x10^{-6}$	$1.3x10^{-9}$	Nitrofurantoin tablet, Tap water, Pond water	[45]
MWCNTs/CS/Au/GCE	Hydroquinone and catechol	DPV	$0.5x10^{-6}$ - $1.5x10^{-3}$ (HQ) $5\ x10^{-6}$ - $0.9\ x10^{-3}$ (CT)	$0.17x10^{-6}$(HQ) $0.89x10^{-6}$(CT)	Tap wate	[53]
GO-CS/Pt	Hydrazine	AM	$2.0x10^{-5}$ - $1.0x10^{-2}$	$3.6x10^{-6}$	Industrial waste water	[54]
NiMoO4/CS/GCE	Amlodipine	DPV	$0.1\ x10^{-6}$ - $374.5x10^{-6}$	$12.74x10^{-9}$	Pharmaceutical Formulation and Human Serum	[40]
AuNPs-GO-CS-ECH/GCE	Clindamycin	SWV	$9.5x10^{-7}$ - $1.4x10^{-4}$	$2.9x10^{-7}$	Pharmaceutical formulations, synthetic urine and river water	[55]
Cs+AuNPs/ SPCE	Aspirin	DPV	$5.5x10^{-12}$ - $5.5x10^{-6}$	$1.6x10^{-13}$	Human physiological fluids and tablets Alassane	[22]
CS-Fe_3O_4/GCE	Bendiocarb	SWV	$4.97x10^{-6}$ - $3.01x10^{-5}$	$2.09x10^{-6}$	Natural raw waters samples	[50]
AuNP/CS/SPE	Ciprofloxacin sensing	SWV	$0.1x10^{-6}$ - $150x10^{-6}$	$0.001x10^{-6}$	Serum, plasma, and urine samples	[52]
Co-CNT-CS/ CPE	Daclatasvir	DPV	$1.0x10^{-9}$- $12x10^{-3}$	$8.82x10^{-10}$	Urine and serum samples	[56]

HANPs: hydroxyapatite nanoparticles; HQ : Hydroquinone ; CT : catechol DPV: Differential pulse voltammetry ; SWV: Square wave voltammetry ; AM : Amperometry; CS : Chitosan ; ECH: Epichlorohydrin; MWCNT : Multiwalled carbon nanotubes ; GO : Graphene oxide ; AuNPs: Gold nanoparticles ; SPE: Screen-printed electrode; GCE: glassy carbon electrode ; CPE : Carbon paste electrode ; CNT : Carbon nanotubes.

3.2.Electrochemical sensors based on chitosan and conducting polymers

Chitosan is a real candidate for the electrochemical sensor construction due to its high mechanical strength, good film-forming ability, high mechanical strength, and high hydrophobicity [57]. But, unfortunately, Chitosan is a non-conductive polymer, for this reason, the researchers combined chitosan with other conductive polymers to increase the charge transfer (Table 2). In fact, chitosan is capable of hybrids polymer formation with different types of conductive polymers having chemical structure conjugated by π-bonds such as polypyrrole, and polyaniline for sensor development (scheme 1) [38,39,58].

The combination of chitosan with different conducting polymers has been reported in the literature, for example, Adeosun *et al.* [38] developed an electrochemical sensor for the detection of sulfite, using

chitosan to facilitate the film formation and polypyrrole to improve the transfer charge. The proposed sulfite sensor had a low LOD and high sensitivity. Furthermore, compared to other established methods such as carbon-coated $NiCo_2O_4$ Nano flower modified glassy carbon electrode [59], chitosan-polypyrrole even showed a lower detection limit. This indicates an improvement in sulfite detection and analysis. A similar chitosan-polypyrrole hybrid polymer in sensor preparation for Pb(II) identification was reported by Xu *et al.*[60]. This electrochemical sensor was successfully used for the determination of Pb(II) in wastewater samples. In the same context, an electrochemical sensor has been prepared for nitrite identification using the drop-casting method of chitosan, polypyrrole, and carboxyl graphene solution on the surface of the glassy carbon electrode. The sensor showed good sensitivity and selectivity for NO^{2-} detection [11].

Another research work was published by Shen et al.[61] consist of the preparation of an electrochemical sensor for the identification of dopamine, using chitosan to facilitate the film formation and poly(3,4-ethylenedioxythiophene) as conducting polymer and graphene to further increase the electronic transfer. The obtained sensor exhibited good sensitivity with a low detection limit of 0.29 µM toward dopamine. Chitosan has been also combined with polyaniline for sensor preparation. In fact, Zad *et al.* [39] developed an electrochemical sensor for the identification of fluconazole using chitosan-polyaniline-Fe_3O_4-Ni-Pd composite. The electrochemical signal was enhanced thanks to the nanoparticles and the conjugation of polyaniline π-bonds. The constructed sensor was effectively applied for the detection of fluconazole in urine, serum, and tablet samples.

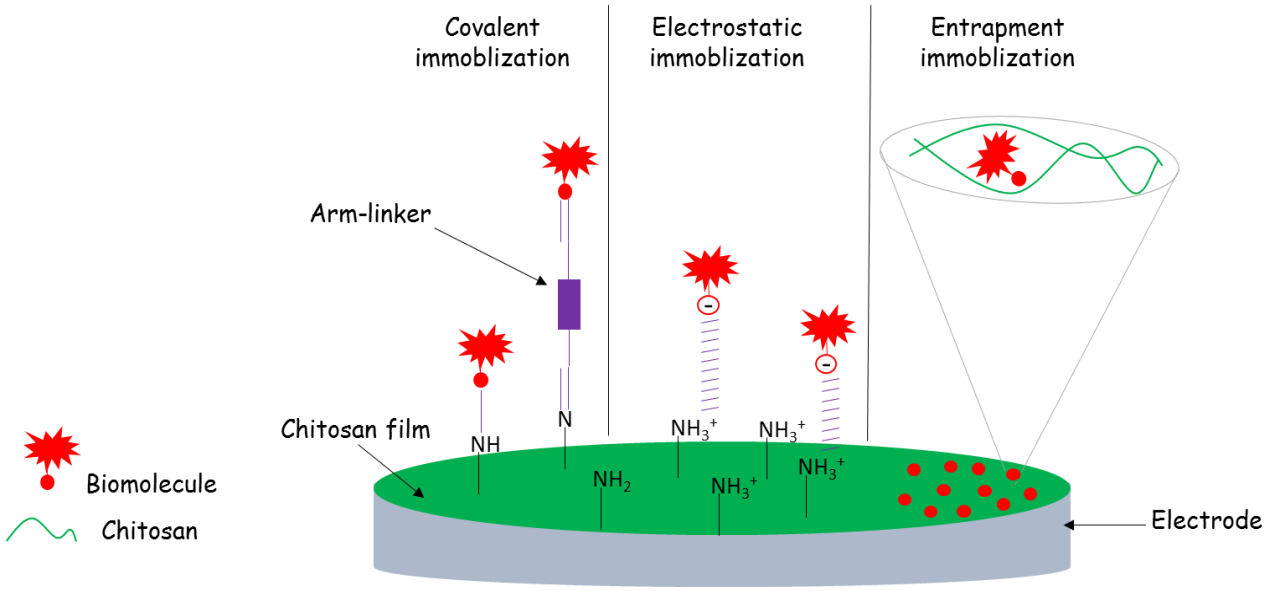
Table 2: Electrochemical sensors based on chitosan-conducting polymers modified electrode.

Electrode	Analyte	Technique	Detection range (mol/L)	LOD (mol/L)	Real sample	Ref
PPY-CS/GCE	sulfite	DPV	$50x10^{-6}$ - $1100x10^{-6}$	$0.21x10^{-6}$	Food and biological samples	[38]
PPy NP/polydopamine/GCE	Pb(II)	DPV	$0.1x10^{-6}$ - $50x10^{-6}$	$55x10^{-9}$	Wastewater	[60]
Poly(VPI+PTZ-(CH2)3SO3−)-CS/Au	Cysteine	DPV	$0.05x10^{-6}$ - $5000x10^{-6}$	$0.06\ x10^{-6}$	--	[62]
PSS/CS–G/SPE	Dopamine	DPV	$0.05\ x10^{-6}$ - $70x10^{-6}$	$0.29x10^{-6}$	--	[61]
Tyr-SPAN-CS/GCE	Phenol	AM	$3.5x10^{-6}$ - $200x10^{-6}$; $20x10^{-8}$ - $2000x10^{-9}$	$0.8x10^{-6}$	Tap water	[63]
Fe3O4@PA-Ni@Pd-Cs/CILE	Fluconazole	DPV	$0.01x10^{-6}$ - $400x10^{-6}$	$3.5x10^{-9}$	Serum, urine, and tablets	[39]
PPy-PDA-CS/GCE	Pb(II)	DPV	$0.1x10^{-6}$ - $50x10^{-6}$	$55x10^{-9}$	Wastewater samples	[60]
CG/PPy/CS/GCE	Nitrite	DPV	$0.2x10^{-6}$ - $1000x10^{-6}$	$0.02x10^{-6}$	Water samples	[11]

CILE: Carbon ionic liquid paste electrod ; PPY: Polypyrrole; GCE: ; Poly(VPI^+PTZ-$(CH2)_3SO_3^-$): Poly(1-vinyl-3-propionate imidazole phenothiazine sulfonic acid ; PSS: poly (styrene sulfonate); SPE: Screen-printed electrode; GCE: Glassy carbon electrode ; G: Graphene; CS; Chitosan; Tyr: Tyrosinase; SPAN: Sulfonated polyaniline; PA: Polyaniline; MWCNTs: Multiwalled carbon nanotubes ; AuNPs: Gold nanoparticles ; DPV : Differential pulse voltammetry ; AM: Amperometry.

4. Electrochemical biosensors based on chitosan

An electrochemical biosensor is a biosensor constituted of an electrode chemically modified, which can provide selective quantitative or semi-quantitative analytical information using a biological recognition element (biomolecule) [64]. The immobilization allows the biomolecules to be fixed and stabilized on the transducer surface. The biomolecules can be immobilized directly on the transducer or on a support such as a chitosan platform that can be rapidly attached to the transducer [65]. Chitosan is an outstanding biopolymer for the development of the electrochemical biosensors because of its relevant chemical, functional, adhesive, and filmogenic properties (Table 3) [8]. Besides, this biocompatible polymer is rich in hydroxyl (OH) and amine (NH_2) groups. Thanks to this later, it can be an excellent candidate for the immobilization of biomolecules. In fact, these reactive groups can be rapidly coupled with various types of biomolecules Such as DNA, enzymes, and antibody/antigen using covalent, electrostatic, or entrapment approaches as described in Scheme 2.



Scheme 2: Chitosan-supported biomolecules immobilization on electrode surfaces.

4.1. Covalent immobilization of biomolecules

Chitosan is widely used as a covalent immobilizing agent of several biomolecules for the development of biosensors. In fact, the chitosan chain is very rich in free amino groups which are highly reactive under mild conditions with carbonyl groups of biomolecules forming very strong imine or amides bonds (Scheme 2), thus explaining the high stability and reproducibility of the biosensors.

Kumar-Krishnan *et al.*[12] developed a novel sensitive glucose biosensor using chitosan film as an immobilization platform for glucose oxidase enzyme and silver nanowires to enhance the electronic transfer rate. The glucose oxidase was immobilized on the chitosan via covalent bonds, which protected the enzyme to remain stable while retaining its activities and ensuring an efficient electrical connection.

The resultant biosensor exhibits good sensitivity, selectivity, and long-term stability for glucose determination. In the same context, Kumar Krishnan et al.[66] published another research work for the detection of glucose using Pd@Pt nanocrystals to facilitate electron transfer, and chitosan for the immobilization of glucose oxidase by covalent linkage through active amino ($-NH_2$) side groups to improve the stability. The biosensor provides remarkable opportunities for designing low-cost and sensitive biosensors toward glucose.

Several reports related to the chitosan platform for covalent immobilization of DNA using the arm-linker molecules such as glutaraldehyde. In fact, Gayathri *et al.*[67] prepared an electrochemical biosensor for the identification of the aromatic compounds. The glutaraldehyde has been used to link covalently between chitosan and DNA nanostructures by forming imine bonds between the aldehyde (-HCO) groups of glutaraldehyde and the amine groups ($-NH_2$) of chitosan and DNA nanostructures. The biosensor was effectively used for the detection of the aromatic compounds. Xu *et al.*[68] developed an electrochemical biosensor based on covalent immobilization of probe DNA on fern leaf-like α-Fe_2O_3 and chitosan hybrid film using terephthalaldehyde as arm-linker. The biosensor exhibited good selectivity and sensitivity for the target DNA.

Chitosan has been used also for the elaboration of electrochemical immunosensors. Because of its ability to fix the antibodies by covalent binding between amino groups of chitosan and the immunoglobins employing, for example, Xiang *et al.*[69] developed a sensitive electrochemical immunosensor for the salmonella detection. The composite film of chitosan-gold nanoparticles functionalized by carbonyl groups was employed for the immobilization of capture antibody (Ab1) for biorecognition. After the fixation of salmonella and horseradish peroxidase (HRP) conjugated secondary antibody (Ab2) a sandwich electrochemical immunosensor has been obtained. The authors reported that immunosensor was effectively applied for the detection of salmonella. Ma *et al.*[14] prepared a novel electrochemical biosensor based on one-step electrodeposition of chitosan-gold nano-composite on gold microelectrode for the aflatoxin B1 identification. The film nano-composite offers abundant amine groups for covalently antibody fixation. The simple method presented good fabrication controllability and high affinity of aflatoxin. Another research work was published by Amit *et al* [70] consist in the preparation of a label-free amperometric biosensor based on chitosan-Y_2O_3 nanocomposite for the determination of norfloxacin. The fluoroquinolones antibodies were immobilized onto chitosan nanocomposite via covalent interactions.

The glutaraldehyde is used largely as a cross-linking reagent to link covalently between chitosan and antibodies. Indeed, Güner *et al.*[71] prepared a novel electrochemical immunosensor for the Escherichia coli detection, using Chitosan, Polypyrrole, Multiwalled carbon nanotubes with gold nanoparticles

hybrid sensing platform. The glutaraldehyde has been employed to link between chitosan and antibody by forming imine bonds between the aldehyde (-HCO) groups of glutaraldehyde and the amine groups (-NH_2) of chitosan and the monoclonal anti-*E. coli* O157:H7. The immunosensor showed good reproducibility and stability for the application in in food quality and safety control.

4.2.Electrostatic immobilization of biomolecules

Chitosan has demonstrated its capability to be used as a good biocompatible platform for electrostatic immobilization of biomolecules including DNA, Enzymes, and antibodies/antigen [72–74]. Indeed, in acidic media, the amino groups (–NH_2) of chitosan are transformed into ammonium groups (–NH_3^+) [75], which facilitates the electrostatic interaction between the chitosan matrix and the negatively charged groups of the desired biomolecule.

In this context, Anusha et al.[73] developed a novel amperometric biosensor using chitosan nanoparticles as an immobilization platform for glucose oxidase enzyme and glucose as substrate. The immobilization was carried out by electrostatic interaction between positively charged of amino groups of chitosan nanoparticles and negatively charged glucose oxidase. The biosensor exhibited high sensitivity and excellent substrate affinity towards the enzyme, as well as demonstrated good reproducibility, repeatability, and stability. Similar chitosan function in biosensor preparation for glucose identification was reported by Nazemi *et al.*[23] The electrostatic immobilization method was applied for the fixation glucose oxidase enzyme onto the chitosan platform. Therefore, the authors were able to develop a novel biosensor with good sensitivity and selectivity for the determination of glucose.

Recently, Mendes et al.[76] prepared an electrochemical biosensor based on laccase immobilized on a nanocomposite chitosan-ZnO nanoparticles. The lactase enzyme was fixed using electrostatic interaction through the -COO^- groups of amino acids located at the surface of the protein, with free ammonium groups (–NH_3^+) of the chitosan layer. The biosensor was successfully used for the determination of Chlorophenol in industrial wastewater.

Chitosan has also applied as an immobilization matrix for DNA using electrostatic binding. The latter depending on the attraction between cationic amino groups in the chitosan chain and anionic phosphate groups in the backbone of the DNA chains. Indeed, Majumdar *et al.*[77] developed an electrochemical biosensor for the detection of nitrosamines. The DNA was electrostatically immobilized on chitosan carbon dots composite. The authors reported that the biosensor was successfully used for the determination of *N*-nitrosodimethylamine and *N*-nitrosodiethanolamine.

Another work was published by Gu *et al.*[78] that consists in the development of a new electrochemical biosensor for the detection of polycyclic organic compounds using the protonated amino groups of chitosan to immobilize electrostatically the DNA. The prepared biosensor exhibited good sensitivity and

selectivity toward polycyclic organic compounds. The immobilization of DNA performance can also be enhanced by chitosan nanoparticles [13]. Indeed, the electrostatic interaction between the free positive charged amino groups of chitosan nanoparticles and the negatively charged phosphate groups of the DNA ensures stable immobilization. The authors reported that the biosensor was effectively used for the determination of toxic metal ion Hg^{2+} in water samples [13].

The chitosan platforms have also been applied for the electrostatic immobilization of antibodies. For example, Singh and colleagues fabricated an electrochemical immunosensor based chitosan-$Ni_3V_2O_8$ composite for biomonitoring of cardiac troponin I. The cationic amine ($-NH_3^+$) groups present in chitosan chain facilitated their binding with carboxyl ($-COO^-$) groups of the cardiac antibody of troponin I via electrostatic interaction. A good sensitivity, stability, and selectivity were achieved [74].

4.3.Entrapment immobilization of biomolecules

Hydrogels of chitosan are largely applied for the immobilization of several biomolecules using the entrapment method. This property could be very useful for the elaboration of electrochemical biosensors. In this context, Burrs *et al*.[79] Prepared a chitosan hydrogel for the encapsulation of alcohol oxidase enzyme and then coated onto a nanoplatinum-graphene modified electrode. The biosensor was effectively applied for the catalytic oxidation of methanol to produce hydrogen peroxide. The authors reported that the chitosan hydrogel biosensor had the highest sensitivity, electro-active surface, and the fastest response time. Another work was for the detection of phenol [63]. The tyrosinase enzyme was immobilized on the electrode surface by entrapment in a chitosan-sulfonated polyaniline composite. The biosensor exhibited a good selectivity and sensitivity with low detection limit of 0.8 nM.

Currently, there is an increasing number of electrochemical biosensors that apply chitosan as an enzyme immobilization matrix using the entrapment method, Stoytcheva *et al.*[80] fabricated a new amperometric bi-enzyme sensor for the organophosphorus pesticides determination using multi-walled carbon nanotubes to enhance the electron transfer rate, and chitosan matrix to entrap the organophosphorus hydrolase and horseradish peroxidase enzymes. The main advantage of this biosensor is its high sensitivity and selectivity toward the organophosphorus pesticides.

An electrochemical sensor for the determination of glucose was fabricated by Senel *et al.*[81] using in situ electropolymerization of pyrrole and thiophene-grafted chitosan (Ch-PTh-PPy). The glucose oxidase was entrapped into the Ch-PTh-PPy layer. The authors reported that such biosensor is promising in biosensor technology due to its electrical conductivity and its biocompatibility. Another research work was published by Liu *et al.*[82] consist in the development of an electrochemical biosensor for the identification of glucose, using chitosan film for the entrapment glucose oxidase. The biosensor exhibited good sensitivity and selectivity for glucose.

Table 3: Typical chitosan platforms for electrochemical biosensing.

Electrode	Biomolecule	Analyte	Immobilization method	Technique	Detection range (mol/L)	LOD (mol/L)	Real sample	Ref
GOx-CS-PTh-PPy/ PGE	Glucose oxidase	Glucose	Entrapment	AM	$1x10^{-3}$ - $8x10^{-3}$	$90x10^{-6}$	--	[81]
ZnO /CS/CPE	Lactase enzyme	Chlorophenol	Electrostatic	DPV	10^{-6} - 50 $x10^{-6}$	$0.7x10^{-6}$	Industrial wastewater	[76]
GOx/ZnONPs-IL/CS/GCE	Glucose oxidase	Glucose	Covalent	CV	10^{-11} - $0.5x10^{-3}$	10^{-12}	--	[83]
CS-GOx	Glucose oxidase	Glucose	Entrapment	CV	0 - 10 $x10^{-3}$	--	--	[82]
CSNPs-GOx/Au	Glucose oxidase	Glucose	Electrostatic	AM	$0.001x10^{-3}$ - 10^{-3}	$1.1x10^{-6}$		[73]
AOx-CS	Alcohol oxidase	Methanol	Entrapment	DCPA	100 $x10^{-6}$ - $2500x10^{-6}$	$100x10^{-6}$	--	[79]
CS-CAT/β-CD-FE	Catalase	H_2O_2	Covalent	chronoamperometric	10^{-7}- $6.0x10^{-3}$	5×10^{-8}	--	[84]
CS-GLM/GCE	Glucose oxidase	Glucose	Entrapment	CV	$0.01x10^{-3}$ -$10x10^{-3}$	$1.32x10^{-6}$	Orange and apple juice	[85]
Pd-Pt NCs-CS-GOx/GCE	Glucose oxidase	Glucose	Covalent	CV	10^{-3}- $6x10^{-3}$	$0.2x10^{-6}$	--	[66]
OPH-HRP bi-enzyme	Organophosphorus hydrolase and horseradish peroxidas	Organophosphorus Pesticides	Entrapment	AM	$2.6x10^{-6}$ - $35x10^{-6}$	$0.8x10^{-6}$	--	[80]
Au/CS/RGO/GCE	Cytochrome P450 bienzyme	Clopidogrel	Covalent	CV	$2x10^{-6}$ - $50x10^{-6}$	$0.63x10^{-6}$	--	[86]
Tyr-SPAN-CS/GCE	Tyrosinase enzyme	Phenol	Entrapment	AM	$3.5x10^{-9}$-$200x10^{-9}$; $200x10^{-9}$ - $2000x10^{-9}$	$0.8x10^{-6}$	Tap water	[63]
SC-HRP/GCE	Horseradish peroxidase	Hydrogen peroxide	Entrapment	CV	$5x10^{-6}$ - $40x10^{-6}$	$5x10^{-6}$	--	[87]
NB/GOx/CS/ION P/AP/GCE	Glucose oxidase	Glucose	Electrostatic	AM	$0.019x10^{-6}$ - $8.6x10^{-3}$	$19x10^{-6}$	--	[23]
CS-GRO/GCE	DNA	DNA	Electrostatic	DPV	1.7–80ppm	0.78ppm	--	[88]
DNA/CSCD/GCE	DNA	Nitrosodimethylamine (NDMA) N-Nitrosodiethanolamine (NDEA)	Electrostatic	DPV	$9.9x10^{-9}$ - $7.4x10^{-7}$ (NDMA); $9.6x10^{-9}$- $4x10^{-7}$ (NDEA)	9.9×10^{-9} (NDMA); 9.6×10^{-9} (NDEA)	--	[77]
nsDNA/GTA/CS-MWCNT/GE	DNA	Aromatic compounds	Covalent	EIS	--	--	Effluent of dye and industry of a tannery	[67]
cDNA-MB-CS/GCE	DNA	Tetracycline hydrochloride	Electrostatic	DPV	0-$2.50x10^{-3}$	5 $x10^{-6}$	--	[78]
ds-DNA-MWNTs-TiO_2/ZrO_2^- CS/ PE	DNA	Taxol	Electrostatic	DPV	$0.7x10^{-9}$ - $1874x10^{-9}$	$0.01x10^{-9}$	Serum, urine and Taxol injection solution samples	[72]
S2-CP-AuNP/S1/CS–GR/GCE	DNA	p53 gene	Covalent	DPV	10^{-15} - 10^{-9}	$3.0x10^{-16}$	--	[89]

S1/TPA/CS-Fe_2O_3/GCE	DNA	DNA	Covalent	EIS	1.0×10^{-14} - 1.0×10^{-10}	5.6×10^{-15}	--	[68]
CS@3D-rGO@DNA	DNA	Hg(II)	Electrostatic	EIS	0.1 $x10^{-9}$ - $10x10^{-9}$	$0.016x10^{-9}$	Tap and river water	[13]
PPy/AuNP/MWCNT@CS	Monoclonal anti-*E. coli* O157:H7	Escherichia coli O157:H7	Covalent	CV	$3 \times 10^{1} – 3 \times 10^{7}$ cfu/mL	30 cfu/mL	Food samples	[71]
BSA/anti-FQ/CS-Y_2O_3/ITO	Fluoroquinolones antibodies	Norfloxacin	Covalent	DPV	10^{-12} - $10x10^{-6}$	$3.87x10^{-12}$	Human urine samples	[70]
CS-AuNPs/Au	anti-Aflatoxin B1	Aflatoxin B1	Covalent	DPV	$3.2x10^{-10}$ - $3.2x10^{-9}$; $3.2x10^{-9}$ - $9.6x10^{-8}$	$1.9x10^{-10}$	Maize	[14]
cAb/CS-Ni3V2O8/Au	Cardiac antibody of troponin I	Cardiac troponin I	Electrostatic	CV	$2.09x10^{-13}$– $4.18x10^{-9}$	$2.09x10^{-13}$	Serum sample	[74]
Gold nanodendrites/CS/	Polyclonal antibody	Botulinum neurotoxin A	Covalent	EIS	0.2–230 pg/mL	0.15 pg/mL	Milk and serum samples	[90]
PAADs@CNDs@Ab2	Anti-Alpha-fetoprotein	Alpha-fetoprotein	Covalent	ECL	$1.4x10^{-17}$ - $1.14x10^{-9}$	$4.7x10^{-18}$	Human serum samples	[91]

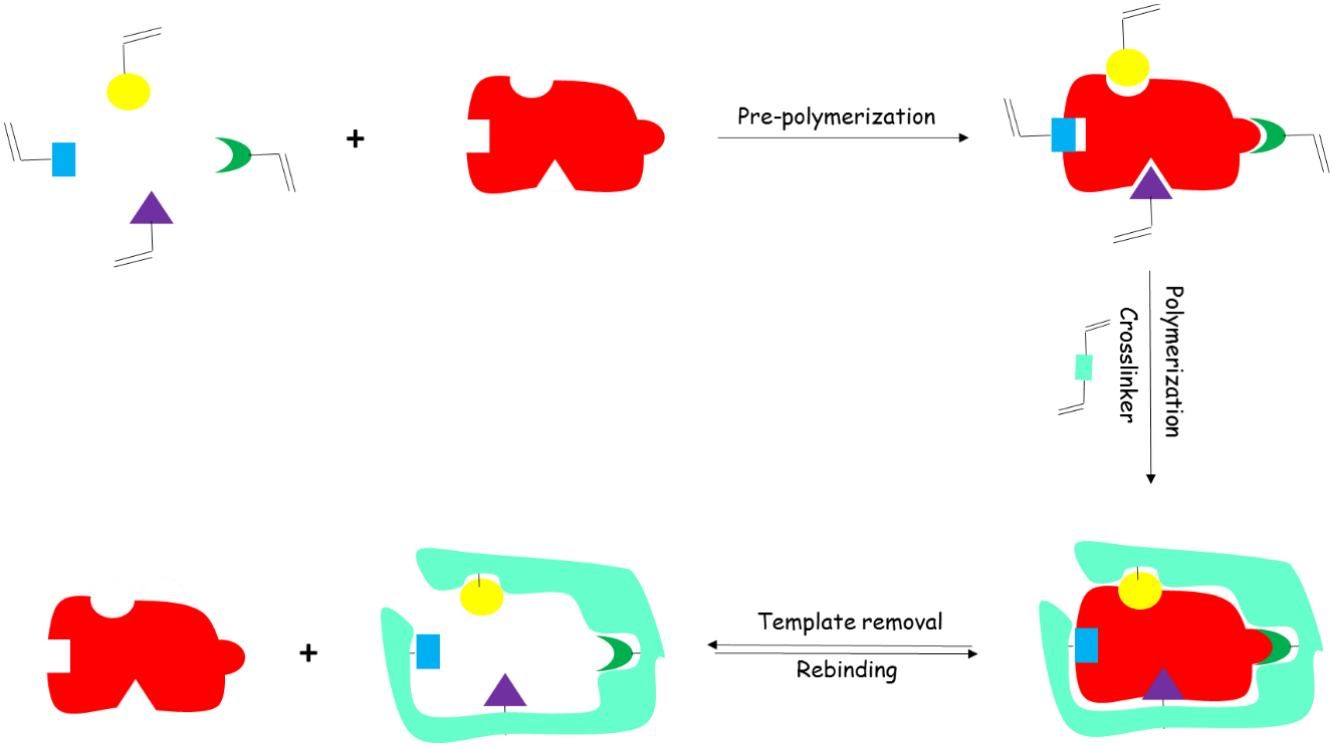
CS : Chitosan ; PTh : Poly(thiophene); DCPA : DC potential amperometry ; PGE: Pencil graphite electrode; CPE: Carbon paste electrode;GOx: Glucose oxidase; ZnONPs : ZnO nanoparticles; IL: Ionic liquid ; GCE: Glasy carbone electrode ; Au :Gold ;CSNPs : Chitosan nanoparticles ; AOx : Alcohol oxidase ; CAT : Catalase ; β-CD : β-cyclodextrin ; FE : Ferrocene ;GOx : Glucose oxidase ; GLM : Glucose oxidase liposome microreactor ; NCs : Metal nanocrystals ; RGO : Reduced graphene oxide ; Tyr: Tyrosinase; SPAN: Sulfonated polyaniline ; HRP : Horseradish peroxidase ; CSCD : Chitosan carbon dots ; GTA : Glutaraldehyde ; GE : Graphite electrode ; MB : Methylene blue ; PE : Pencil electrode ; MWNTs : Multiwalled carbon nanotubes ; S1 : probe sequence ; S2 : Complementary sequence; CR : graphene ; AuNPs : Gold nanoparticles ; NB : Nile blue; IONP: Iron oxide nanoparticles ; AP : Aminophenyl modified ; TPA : Terephthalaldehyde ; 3D-rGO three-dimensional reduced graphene oxide ; BSA :Bovine serum albumin ; ITO : Indium Tin Oxide ; Anti-FQ : Fluoroquinolones antibodies ; cAb : Cardiac antibody of troponin I ; PAADs :Poly(amidoamine) dendrimers ; CNDs : Carbon nanodots.

5. Molecularly imprinted chitosan-based electrochemical sensors

MIP is a synthetic material obtained by the polymerization reaction of a functional monomer in the presence of a template and a cross-linking agent. After the extraction step, MIP contains specific cavities for a target molecule (Scheme 3). The manner of binding MIP to the target molecule is the same as that of antibody/antigen [92]. MIPs present various advantages such as fast preparation, low cost, and chemical stability [93,94].

The richness of the chitosan chain in amino and hydroxyl groups facilitates its reaction with several types of cross-linking agents (epoxides, ketones, aldehydes, and acid groups). This cross-linking reaction allows the creation of specific cavities for the target molecules. Besides, thanks to the chitosan biocompatibility and film-forming ability its MIPs are widely employed as receptors for the elaboration of sensors (Table 4). A catechol electrochemical sensor was prepared by Salvo-Comino *et al*.[95] using chitosan as imprinting polymer, gold nanoparticles to enhance electronic transfer, and catechol as a template molecule. The obtained solution was deposited onto the boron-doped diamond electrode surface via the drop-costing method. The fabricated sensor was effectively used for the detection of catechol in a real wine sample. Another research work was published by Gan *et al.*[96] consist in the

development of an electrochemical sensor based on a multiwall carbon nanotube, SnS_2, and molecularly imprinted chitosan for the identification 6-benzylaminopurine. The authors reported that the sensor has high sensitivity and selectivity toward 6-benzylaminopurine.



Scheme 3: Schematic procedure for the preparation of the molecularly imprinted polymer.

In the same context, Lin *et al*. [97] developed an electrochemical sensor based on molecularly imprinted chitosan for the detection of L-dopa. The sensor was prepared by the electrodeposition of the chitosan-graphen solution onto the glassy carbon electrode surface. This electrochemical sensor exhibited good sensitivity and selectivity toward L-dopa in pharmaceutical preparations and human blood serum. A molecularly imprinted electrochemical quartz crystal microbalance sensor for the determination of aspartame was developed by Srivastava *et al*. [98] by grafting aspartame-imprinted chitosan nanoparticles on gold-coated quartz crystal electrode. The sensor exhibited high sensitivity and selectivity toward aspartame.

Recently, an ion imprinting chitosan electrochemical sensor was prepared by Wei *et al.* [99] for the Cu (II) identification. The sensor fabricated by chitosan-graphene oxide composite modified glassy carbon electrode. The obtained sensor presented high sensitivity and selectivity for the detection of Cu(II) in tap and river water samples. Another chitosan-based electrochemical sensor was developed for the determination of bisphenol A [100]. The chitosan imprinting polymer was mixed with bisphenol A and then electrodeposited onto the surface of a gold electrode. The sensor was effectively used for the identification of bisphenol A in real plastic bottles for drinking water.

Table 4: Electrochemical sensors based on molecularly imprinted chitosan.

Analyte	MIP/Electrode	Technique	Linear range (mol/L)	LOD (mol/L)	Matrix	Ref
Catechol	CS/BDD	CV	10^{-6} - 80×10^{-6}	6.9×10^{-7}	Red wine	[101]
L-5-hydroxytryptophan	GR-MIPs/GCE	DPV	5×10^{-8} - 7×10^{-6}	6×10^{-9}	Human blood serum	[102]
Dopamine	MIPs-$CuCo_2O_4$/C	DPV	5.1×10^{-4} - 95×10^{-3}	1.6×10^{-7}	-	[103]
Propranolol	MIPs/rGO/GCE	DPV	50×10^{-6} - 10^{-3}	-	Racemic solution	[104]
Bisphenol A	MIPs-AuNPs/GCE	DPV	5.1×10^{-8} - 5.5×10^{-5}	1.1×10^{-9}	Plastic and milk	[105]
Cr(VI)	CS-IIPs	DPV	10^{-9} - 10^{-5}	6.4×10^{-10}	Tap water and river water	[106]
Bisphenol A	MIC/Au	SWV	10^{-3} - 10^{-21}	0.67×10^{-21}	Plastic bottles for drinking water	[100]
Catechol	CS-CNT-AuNPs/BDD	CV	75×10^{-6} –0.001	3.7×10^{-5}	Red wine	[95]
L-dopa	GR-MIPs/GCE	DPV	4×10^{-7} - 10^{-4}	1.2×10^{-8}	Tablet and Human blood serum	[97]
Aspartame	CSNP–rGO	EQCM/DPV	10^{-5} - 10^{-4}	1.5×10^{-6} (EQCM), 2.3×10^{-7}(DPV)	Human blood plasma	[98]
Cu(II)	CS-GO/GCE	DPASV	5×10^{-7}-$\times10^{-4}$	0.15 10^{-6}	Tap and river water samples	[99]
Glyphosate	CS-SO_4^{2-}/Au	EIS	1.83×10^{-12} - 2.9×10^{-7}	5.9×10^{-15}	River water	[107]
6-Benzylaminopurine	MIP-MWCNT-SnS_2/GCE	DPV	10^{-10}–10^{-2}	50×10^{-12}	Soybean sprout, mung bean sprout, potato, tomato, pear, and apple	[96]
Tryptophan	MIP-MWCNTs/GCE	SDLSV	2×10^{-9}- 2×10^{-7}, 2×10^{-6} - 10^{-5}, 10^{-5} - 10^{-4}	1×10^{-9}	Human serum	[108]
Bisphenol A	CS-AB/GCE	SWV	5×10^{-9} - 2×10^{-7}, 5×10^{-7} - 10^{-5}	2×10^{-9}	Drinking water	[29]

Au gold electrode, CS chitosan, MWCNTs multi-walled carbon nanotubes, AuNPs gold nanoparticles, GR reduced graphene, CNT carbon nano- tubes, GCE glassy carbon electrode, GO graphene oxide, IIPs ions imprinted polymers, MIC molecularly imprinted chitosan, MIPs molecularly imprinted polymers, BDD boron doped diamond electrode, AB acetylene black, rGO reduced graphene oxide, CSNP chitosan nanoparticle, DPV differential pulse voltammetry, CV cyclic voltammetry, SWV square wave voltammetry, EIS electrochemical impedance spectroscopy, EQCM electrochemical quartz crystal microbalance, SDLSV second-order derivative linear sweep voltammetry.

6. Conclusions and future prospects

According to the results reported above, chitosan is considered an excellent candidate for the development of the electrochemical sensors and biosensors. Indeed, the combination of chitosan with nanoparticles and conductive polymers have provided a sensitive determination of the analytes regarding their high surface area and high electron transfer. Also, chitosan has proven its efficiency to be employed as an immobilization platform for biomolecules using covalent, electrostatic, or entrapment approaches. Moreover, the molecularly imprinted chitosan-based electrochemical sensors have shown good stability, reproducibility, and high sensitivity towards several types of analytes.

Recent trends imply the application of nanoparticles of chitosan for the development of biosensors in order to increase the specific surface area as well as the number of free amine functions responsible for the immobilization of biomolecules. Also, the modification of nanoparticles such as carbon nanotubes

and gold nanoparticles by chitosan will provide novel nanocomposites. These latter will possess amine and hydroxyl groups capable to immobilize several types of biomolecules and also, they will exhibit a good conductivity and a large specific surface area. Thanks to their properties, these nanocomposites will be excellent candidates for the development of sensors and biosensors.

Conflict of Interest-The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

[1] R.K. Majeti N.V, A review of chitin and chitosan applications, *React. Funct. Polym.* 46: 1–27 (2000). https://doi.org/10.1016/S1381-5148(00)00038-9.

[2] B. Qu, Y. Luo, Chitosan-based hydrogel beads: Preparations, modifications and applications in food and agriculture sectors – A review, *Int. J. Biol. Macromol.* 152: 437–448 (2020). https://doi.org/10.1016/j.ijbiomac.2020.02.240.

[3] H.K. No, S.P. Meyers, W. Prinyawiwatkul, Z. Xu, Applications of chitosan for improvement of quality and shelf life of foods: A review, *J. Food Sci.* 72: (2007). https://doi.org/10.1111/j.1750-3841.2007.00383.x.

[4] C. Shi, Y. Zhu, X. Ran, M. Wang, Y. Su, T. Cheng, Therapeutic Potential of Chitosan and Its Derivatives in Regenerative Medicine1 1 This work was supported by "973" programs on severe trauma (NO. 1999054205 and NO. 2005CB522605) from the Ministry of Science and Technology of China., *J. Surg. Res.* 133: 185–192 (2006). https://doi.org/10.1016/j.jss.2005.12.013.

[5] D. Enescu, Use of chitosan in surface modification of textile materials, *Rom. Biotechnol. Lett.* 13: 4037–4048 (2008).

[6] R. Yang, H. Li, M. Huang, H. Yang, A. Li, A review on chitosan-based flocculants and their applications in water treatment, *Water Res.* 95: 59–89 (2016). https://doi.org/10.1016/j.watres.2016.02.068.

[7] A. Karrat, A. Lamaoui, A. Amine, J. María, P. Santander, Applications of Chitosan in Molecularly and Ion Imprinted Polymers, *Chem. Africa*. 1–21 (2020). https://doi.org/10.1007/s42250-020-00177-w.

[8] W. Suginta, P. Khunkaewla, A. Schulte, Electrochemical biosensor applications of polysaccharides chitin and chitosan, Chem. Rev. 113: 5458–5479 (2013). https://doi.org/10.1021/cr300325r.

[9] U. Guth, W. Vonau, J. Zosel, Recent developments in electrochemical sensor application and technology - A review, *Meas. Sci. Technol.* 20: (2009). https://doi.org/10.1088/0957-0233/20/4/042002.

[10] M. Baccarin, F.A. Santos, F.C. Vicentini, V. Zucolotto, B.C. Janegitz, O. Fatibello-Filho, Electrochemical sensor based on reduced graphene oxide/carbon black/chitosan composite for the simultaneous determination of dopamine and paracetamol concentrations in urine samples, *J. Electroanal. Chem.* 799: 436–443 (2017). https://doi.org/10.1016/j.jelechem.2017.06.052.

[11] Q. Xiao, M. Feng, Y. Liu, S. Lu, Y. He, S. Huang, The graphene/polypyrrole/chitosan-modified glassy carbon electrode for electrochemical nitrite detection, *Ionics (Kiel).* 24: 845–859 (2018). https://doi.org/10.1007/s11581-017-2247-y.

[12] S. Kumar-Krishnan, S. Chakaravarthy, A. Hernandez-Rangel, E. Prokhorov, G. Luna-Bárcenas, R. Esparza, M. Meyyappan, Chitosan supported silver nanowires as a platform for direct electrochemistry and highly sensitive electrochemical glucose biosensing, *RSC Adv.* 6: 20102–20108 (2016). https://doi.org/10.1039/c5ra24259b.

[13] Z. Zhang, X. Fu, K. Li, R. Liu, D. Peng, L. He, M. Wang, H. Zhang, L. Zhou, One-step fabrication of electrochemical biosensor based on DNA-modified three-dimensional reduced graphene oxide and chitosan nanocomposite for highly sensitive detection of Hg(II), *Sensors Actuators, B Chem.* 225 (2016) 453–462. https://doi.org/10.1016/j.snb.2015.11.091.

[14] H. Ma, J. Sun, Y. Zhang, C. Bian, S. Xia, T. Zhen, Label-free immunosensor based on one-step electrodeposition of chitosan-gold nanoparticles biocompatible film on Au microelectrode for determination of aflatoxin B1 in maize, *Biosens. Bioelectron.* 80: 220-229 (2016). https://doi.org/10.1016/j.bios.2016.01.063.

[15] L. Figueiredo, G.L. Erny, L. Santos, A. Alves, Applications of molecularly imprinted polymers to the analysis and removal of personal care products: A review, *Talanta.* 146: 754–765 (2016). https://doi.org/10.1016/j.talanta.2015.06.027.

[16] V. Zargar, M. Asghari, A. Dashti, A Review on Chitin and Chitosan Polymers: Structure, Chemistry, Solubility, Derivatives, and Applications, *ChemBioEng Rev.* 2: 204–226 (2015). https://doi.org/10.1002/cben.201400025.

[17] K. Kurita, Chitin and chitosan: Functional biopolymers from marine crustaceans, *Mar. Biotechnol.* 8: 203–226 (2006). https://doi.org/10.1007/s10126-005-0097-5.

[18] S.H. Chang, H.T.V. Lin, G.J. Wu, G.J. Tsai, pH Effects on solubility, zeta potential, and correlation between antibacterial activity and molecular weight of chitosan, *Carbohydr. Polym.* 134: 74–81 (2015). https://doi.org/10.1016/j.carbpol.2015.07.072.

[19] B. Carreño-Gómez, R. Duncan, Evaluation Of the biological properties of soluble chitosan and chitosan microspheres, *Int. J. Pharm.* 148: 231–240 (1997). https://doi.org/10.1016/S0378-5173(96)04847-8.

[20] I. Aranaz, N. Acosta, C. Civera, B. Elorza, J. Mingo, C. Castro, M. de los L. Gandía, A.H. Caballero, Cosmetics and cosmeceutical applications of chitin, chitosan and their derivatives, *Polymers* (Basel). 10: (2018). https://doi.org/10.3390/polym10020213.

[21] M. Ahmad, K. Manzoor, S. Ikram, Chitosan based nanocomposites for drug, gene delivery, and bioimaging applications, *Applications of Nanocomposite Materials in Drug Delivery Woodhead Publishing Series in Biomaterials* 27-38 (2018). https://doi.org/10.1016/B978-0-12-813741-3.00002-9.

[22] A. Diouf, M. Moufid, D. Bouyahya, L. Österlund, N. El Bari, B. Bouchikhi, An electrochemical sensor based on chitosan capped with gold nanoparticles combined with a voltammetric electronic tongue for quantitative aspirin detection in human physiological fluids and tablets, *Mater. Sci. Eng. C.* 110: 110665 (2020). https://doi.org/10.1016/j.msec.2020.110665.

[23] Z. Nazemi, E. Shams, M.K. Amini, Construction of a biointerface for glucose oxidase through diazonium chemistry and electrostatic self-assembly technique, *J. Solid State Electrochem.* 20: 429–438 (2016). https://doi.org/10.1007/s10008-015-3045-y.

[24] J.J. Wang, Z.W. Zeng, R.Z. Xiao, T. Xie, G.L. Zhou, X.R. Zhan, S.L. Wang, Recent advances of chitosan nanoparticles as drug carriers., *Int. J. Nanomedicine.* 6: 765–774 (2011). https://doi.org/10.2147/ijn.s17296.

[25] A.A. Tayel, M.M. Gharieb, H.R. Zaki, N.M. Elguindy, Bio-clarification of water from heavy metals and microbial effluence using fungal chitosan, *Int. J. Biol. Macromol.* 83: 277–281 (2016). https://doi.org/10.1016/j.ijbiomac.2015.11.072.

[26] I. Hamed, F. Özogul, J.M. Regenstein, Industrial applications of crustacean by-products (chitin, chitosan, and chitooligosaccharides): A review, *Trends Food Sci. Technol.* 48: 40–50 (2016). https://doi.org/10.1016/j.tifs.2015.11.007.

[27] K. Xing, X. Zhu, X. Peng, S. Qin, Chitosan antimicrobial and eliciting properties for pest control in agriculture: a review, *Agron. Sustain. Dev.* 35: 569–588 (2015). doi.org/10.1007/s13593-014-0252-3.

[28] T.C. Yang, C.C. Chou, C.F. Li, Preparation, water solubility and rheological property of the N-alkylated mono or disaccharide chitosan derivatives, Food Res. Int. 35 (2002) 707–713. https://doi.org/10.1016/S0963-9969(02)00064-9.

[29] Y. Tan, J. Jin, S. Zhang, Z. Shi, J. Wang, J. Zhang, W. Pu, C. Yang, Electrochemical Determination of Bisphenol A Using a Molecularly Imprinted Chitosan-acetylene Black Composite Film Modified Glassy Carbon Electrode, *Electroanalysis.* 28: 189–196 (2016). https://doi.org/10.1002/elan.201500533.

[30] C.A. Oyrton, A.C. Monteiro Jr., Some studies of crosslinking chitosan–glutaraldehyde interaction in a homogeneous system, *Int. J. Biol. Macromol.* 26: 119–128 (1999). doi:10.4183/aeb.2010.521.

[31] P.K. Dutta, S. Tripathi, G.K. Mehrotra, J. Dutta, Perspectives for chitosan based antimicrobial films in food applications, *Food Chem.* 114: 1173–1182 (2009). https://doi.org/10.1016/j.foodchem.2008.11.047.

[32] R. Jayakumar, M. Prabaharan, S. V. Nair, H. Tamura, Novel chitin and chitosan nanofibers in biomedical applications, *Biotechnol. Adv.* 28: 142–150 (2010). https://doi.org/10.1016/j.biotechadv.2009.11.001.

[33] K.A. Janes, M.P. Fresneau, A. Marazuela, A. Fabra, M.J. Alonso, Chitosan nanoparticles as delivery systems for doxorubicin, *J. Control. Release.* 73: 255–267 (2001). https://doi.org/10.1016/S0168-3659(01)00294-2.

[34] C. Zhong, B. Yang, X. Jiang, J. Li, Current Progress of Nanomaterials in Molecularly Imprinted Electrochemical Sensing, *Crit. Rev. Anal. Chem.* 48: 15–32 (2018). https://doi.org/10.1080/10408347.2017.1360762.

[35] Y. Zannatul, Z. Maogen, G. Waldemar, M. Saher, G. Randolph, L.N. Kelly, Rare Earth Nanocomposites Based on Chitosan Platforms for Biological Applications Zannatul, *Mater. Res. Soc. Symp. Proc.* 1477: 61–66 (2012). https://doi.org/10.1557/opl.2012. 1076.

[36] W.T. Wahyuni, Z. Arif, N.S. Maryam, Development of graphene-chitosan composite modified screen-printed carbon electrode and its application for detection of rutin, *IOP Conf. Ser. Earth Environ. Sci.* 299 (2019). https://doi.org/10.1088/1755-1315/299/1/012005.

[37] W. Yu, Y. Tang, Y. Sang, W. Liu, S. Wang, X. Wang, Preparation of a carboxylated single-walled carbon-nanotube-chitosan functional layer and its application to a molecularly imprinted electrochemical sensor to quantify semicarbazide, *Food Chem.* 333: 127524 (2020). https://doi.org/10.1016/j.foodchem.2020.127524.

[38] W.A. Adeosun, A.M. Asiri, H.M. Marwani, Fabrication of Conductive Polypyrrole Doped Chitosan Thin Film for Sensitive Detection of Sulfite in Real Food and Biological Samples, *Electroanalysis.* 1–35 (2020). https://doi.org/10.1002/elan.201900765.

[39] Z.R. Zad, S.S.H. Davarani, A. Taheri, Y. Bide, A yolk shell Fe_3O_4 @PA-Ni@Pd/Chitosan nanocomposite -modified carbon ionic liquid electrode as a new sensor for the sensitive determination of fluconazole in pharmaceutical preparations and biological fluids, *J. Mol. Liq.* 253: 233–240 (2018). https://doi.org/10.1016/j.molliq.2018.01.019.

[40] B. Lou, U. Rajaji, S. Chen, T. Chen, A Simple Sonochemical Assisted Synthesis of Porous NiMoO4/chitosan Nanocomposite for Electrochemical Sensing of Amlodipine in Pharmaceutical

Formulation and Human Serum, *Ultrason. Sonochem.* 1–27 (2019). https://doi.org/10.1016/j.ultsonch.2019.104827.

[41] A. Mohammadi, A.B. Moghaddam, K. Eilkhanizadeh, E. Alikhani, S. Mozaffari, T. Yavari, Electro-oxidation and simultaneous determination of amlodipine and atorvastatin in commercial tablets using carbon nanotube modified electrode, *Micro Nano Lett.* 8: 413–417 (2013). https://doi.org/10.1049/mnl.2013.0080.

[42] Z.Z. Stoiljković, M.L. Avramov Ivić, S.D. Petrović, D.Z. Mijin, S.I. Stevanović, U.C. Lačnjevac, A.D. Marinković, Voltammetric and square-wave anodic stripping determination of amlodipine besylate on gold electrode, *Int. J. Electrochem. Sci.* 7: 2288–2303 (2012).

[43] Y.F. Jin, C.Y. Ge, X.B. Li, M. Zhang, G.R. Xu, D.H. Li, A sensitive electrochemical sensor based on ZIF-8-acetylene black-chitosan nanocomposites for rutin detection, *RSC Adv.* 8: 32740–32746 (2018). https://doi.org/10.1039/c8ra06452k.

[44] S. Akhter, W.J. Basirun, Y. Alias, M.R. Johan, S. Bagheri, M. Shalauddin, M. Ladan, N.S. Anuar, Enhanced amperometric detection of paracetamol by immobilized cobalt ion on functionalized MWCNTs - Chitosan thin film, *Anal. Biochem.* 551: 29–36 (2018). doi10.1016/j.ab.2018.05.004

[45] S. Velmurugan, S. Palanisamy, T.C. Yang, Ultrasonic assisted functionalization of MWCNT and synergistic electrocatalytic effect of nano-hydroxyapatite incorporated MWCNT-chitosan scaffolds for sensing of nitrofurantoin a Department b Precision, *Ultrason. Sonochem.* 104863 (2019). https://doi.org/10.1016/j.ultsonch.2019.104863.

[46] S.M. Patil, S.R. Sataraddi, A.M. Bagoji, R.M. Pathan, S.T. Nandibewoor, Electrochemical behavior of graphene-based sensors on the redox mechanism of aspirin, *Electroanalysis.* 26: 831–839 (2014). https://doi.org/10.1002/elan.201300650.

[47] H. Karimi-Maleh, S. Rostami, V.K. Gupta, M. Fouladgar, Evaluation of ZnO nanoparticle ionic liquid composite as a voltammetric sensing of isoprenaline in the presence of aspirin for liquid phase determination, *J. Mol. Liq.* 201: 102–107 (2015). doi.org/10.1016/j.molliq.2014.10.042.

[48] A. Trani, R. Petrucci, G. Marrosu, D. Zane, A. Curulli, Selective electrochemical determination of caffeine at a gold-chitosan nanocomposite sensor: May little change on nanocomposites synthesis affect selectivity?, *J. Electroanal. Chem.* 788: 99–106 (2017). https://doi.org/10.1016/j.jelechem.2017.01.049.

[49] F. Nazari, S.M. Ghoreishi, A. Khoobi, Bio-based Fe3O4/chitosan nanocomposite sensor for response surface methodology and sensitive determination of gallic acid, *Int. J. Biol. Macromol.* (2020). https://doi.org/10.1016/j.ijbiomac.2020.05.205.

[50] R.C. de Oliveira, C.P. Sousa, T.M. Freire, R.M. Freire, J.C. Denardin, P.B.A. Fechine, H. Becker,

S. Morais, P. de Lima-Neto, A.N. Correia, Chitosan-magnetite nanocomposite as a sensing platform to bendiocarb determination, *Anal. Bioanal. Chem.* 410: 7229–7238 (2018). https://doi.org/10.1007/s00216-018-1330-1.

[51] N. Feng, J. Zhang, W. Li, Chitosan/Graphene Oxide Nanocomposite-Based Electrochemical Sensor For ppb Level Detection of Melamine, *J. Electrochem. Soc.* 166: B1364–B1369 (2019). https://doi.org/10.1149/2.1321914jes.

[52] K.R. Reddy, P.K. Brahman, L. Suresh, Fabrication of high performance disposable screen printed electrochemical sensor for ciprofloxacin sensing in biological samples, *Meas. J. Int. Meas. Confed.* 127: 175–186 (2018). https://doi.org/10.1016/j.measurement.2018.05.078.

[53] Y. Shen, D. Rao, Q. Sheng, J. Zheng, Simultaneous voltammetric determination of hydroquinone and catechol by using a glassy carbon electrode modified with carboxy-functionalized carbon nanotubes in a chitosan matrix and decorated with gold nanoparticles, *Microchim. Acta.* 184: 3591–3601 (2017). https://doi.org/10.1007/s00604-017-2392-z.

[54] D. Rao, Q. Sheng, J. Zheng, Preparation of flower-like Pt nanoparticles decorated chitosan-grafted graphene oxide and its electrocatalysis of hydrazine, *Sensors Actuators, B Chem.* 236: 192–200 (2016). https://doi.org/10.1016/j.snb.2016.05.160.

[55] A. Wong, C.A. Razzino, T.A. Silva, O. Fatibello-Filho, Square-wave voltammetric determination of clindamycin using a glassy carbon electrode modified with graphene oxide and gold nanoparticles within a crosslinked chitosan film, *Sensors Actuators, B Chem.* 231: 183–193 (2016). https://doi.org/10.1016/j.snb.2016.03.014.

[56] S.M. Azab, A.M. Fekry, Electrochemical design of a new nanosensor based on cobalt nanoparticles, chitosan and MWCNT for the determination of daclatasvir: A hepatitis C antiviral drug, *RSC Adv.* 7: 1118–1126 (2017). https://doi.org/10.1039/c6ra25826c.

[57] S. Amjadi, M. Nazari, S.A. Alizadeh, H. Hamishehkar, Multifunctional betanin nanoliposomes-incorporated gelatin/chitosan nanofiber/ZnO nanoparticles nanocomposite film for fresh beef preservation, *Meat Sci.* 167: 108161 (2020). https://doi.org/10.1016/j.meatsci.2020.108161.

[58] Y. Wang, Y. Xiong, J. Qu, J. Qu, S. Li, Selective sensing of hydroquinone and catechol based on multiwalled carbon nanotubes/polydopamine/gold nanoparticles composites, *Sensors Actuators, B Chem.* 223: 501–508 (2016). https://doi.org/10.1016/j.snb.2015.09.117.

[59] K. Pandi, M. Sivakumar, S.M. Chen, Y.H. Cheng, T.W. Chen, Hydrothermal synthesis of carbon coated nico2o4 nano flower for the electrochemical oxidation of sulfite in real sample, *Int. J. Electrochem. Sci.* 13: 1227–1240 (2018). https://doi.org/10.20964/2018.02.27.

[60] T. Xu, H. Dai, Y. Jin, Electrochemical sensing of lead (II) by differential pulse voltammetry

using conductive polypyrrole nanoparticles, Microchim. Acta. 187:23 1–7 (2020). https://doi.org/10.1007/s00604-019-4027-z.

[61] X. Shen, F. Ju, G. Li, L. Ma, Smartphone-based electrochemical potentiostat detection system using pedot: Pss/chitosan/graphene modified screen-printed electrodes for dopamine detection, *Sensors (Switzerland)*. 20 (2020). https://doi.org/10.3390/s20102781.

[62] X. Feng, J. Yi, W. Zhang, Y. Niu, L. Xu, A redox poly (ionic liquid) hydrogel : Facile method of synthesis and electrochemical sensing, *J. Appl. Polym. Sci.* 48051: 1–10 (2019). https://doi.org/10.1002/app.48051.

[63] L. Guo, H. Du, H. Zhao, J. Li, Amplified Electrochemical Response of Phenol by Oxygenation of Tyrosinase Coupling with Electrochemical-chemical-chemical Redox Cycle, *Electroanalysis.* 31: 1728–1735 (2019). https://doi.org/10.1002/elan.201900174.

[64] D.R. Theâvenot, K. Toth, R.A. Durst, G.S. Wilson, Electrochemical Biosensors: Recommended Definitions And Classification., *Pure Appl. Chem.* 71: 2333–2348 (1999).

[65] P. Barathi, B. Thirumalraj, S.M. Chen, S. Angaiah, A simple and flexible enzymatic glucose biosensor using chitosan entrapped mesoporous carbon nanocomposite, *Microchem. J.* 147: 848–856 (2019). https://doi.org/10.1016/j.microc.2019.03.083.

[66] S.K. Krishnan, E. Prokhorov, D. Bahena, R. Esparza, M. Meyyappan, Chitosan-Covered Pd@Pt Core-Shell Nanocubes for Direct Electron Transfer in Electrochemical Enzymatic Glucose Biosensor, *ACS Omega.* 2: 1896–1904 (2017). https://doi.org/10.1021/acsomega.7b00060.

[67] S.B. Gayathri, P. Kamaraj, M. Arthanareeswari, S. Devikala, DNA nanostructures based biosensor for the determination of aromatic compounds, *Biosens. Bioelectron.* 72: 191-196 (2015). https://doi.org/10.1016/j.bios.2015.05.002.

[68] B. Xu, D. Zheng, W. Qiu, F. Gao, S. Jiang, Q. Wang, An ultrasensitive DNA biosensor based on covalent immobilization of probe DNA on fern leaf-like α-Fe_2O_3 and chitosan Hybrid film using terephthalaldehyde as arm-linker, *Biosens. Bioelectron.* 72: 175–181 (2015). https://doi.org/10.1016/j.bios.2015.05.015.

[69] C. Xiang, R. Li, B. Adhikari, Z. She, Y. Li, H. Kraatz, Sensitive electrochemical detection of Salmonella with Chitosan-Gold oparticles composite film, *Talanta.* 140: 122-127 (2015). https://doi.org/10.1016/j.talanta.2015.03.033.

[70] A.K. Yadav, T.K. Dhiman, G.B.V.S. Lakshmi, A.N. Berlina, P.R. Solanki, A highly sensitive label-free amperometric biosensor for norfloxacin detection based on chitosan-yttria nanocomposite, *Int. J. Biol. Macromol.* 151: 566–575 (2020). https://doi.org/10.1016/j.ijbiomac.2020.02.089.

[71] A. Güner, E. Çevik, M. Şenel, L. Alpsoy, An electrochemical immunosensor for sensitive detection of Escherichia coli O157:H7 by using chitosan, MWCNT, polypyrrole with gold nanoparticles hybrid sensing platform, *Food Chem.* 229: 358–365 (2017). https://doi.org/10.1016/j.foodchem.2017.02.083.

[72] M. Taei, F. Hassanpour, H. Salavati, Z. Sadeghi, H. Alvandi, Highly Selective Electrochemical Determination of Taxol Based on ds-DNA-Modified Pencil Electrode, *Appl. Biochem. Biotechnol.* 176: 344–358 (2015). https://doi.org/10.1007/s12010-015-1578-2.

[73] J.R. Anusha, C.J. Raj, B.B. Cho, A.T. Fleming, K.H. Yu, B.C. Kim, Amperometric glucose biosensor based on glucose oxidase immobilized over chitosan nanoparticles from gladius of Uroteuthis duvauceli, *Sensors Actuators, B Chem.* 215: 536–543 (2015). https://doi.org/10.1016/j.snb.2015.03.110.

[74] and R.J. Nawab Singha, Prabhakar Raib, c, Md. Azahar Alid, Rudra Kumarb, Ashutosh Sharmab, B. D. Malhotrae, Hollow-nanospheres-based microfluidic biosensors for biomonitoring of cardiac troponin I, *J. Mater. Chem. B.* 1–31 (2019). https://doi.org/10.1039/C9TB00126C.

[75] J.D. Giraldo, B.L. Rivas, Direct ionization and solubility of chitosan in aqueous solutions with acetic acid, *Polym. Bull.* 1–24 (2020). https://doi.org/10.1007/s00289-020-03172-w.

[76] R.K. Mendes, B.S. Arruda, E.F. De Souza, A.B. Nogueira, O. Teschke, L.O. Bonugli, A. Etchegaray, Determination of chlorophenol in environmental samples using a voltammetric biosensor based on hybrid nanocomposite, *J. Braz. Chem. Soc.* 28: 1212–1219 (2017). https://doi.org/10.21577/0103-5053.20160282.

[77] S. Majumdar, D. Thakur, D. Chowdhury, DNA Carbon-Nanodots based Electrochemical Biosensor for Detection of Mutagenic Nitrosamines, *ACS Appl. Bio Mater.* 3: 1796–1803 (2020). https://doi.org/10.1021/acsabm.0c00073.

[78] Y.J. Tingting GU, Hong-qi XIA, Yue HU, Electrochemical Biosensor for Polycyclic Organic Compounds Screening Based on Methylene Blue Incorporated DNA Polyion Complex Modified Electrode, *Anal. Sci.* 34(10): 1131-1135 (2018). https://doi.org/10.2116/analsci.18P167.

[79] S.L. Burrs, D.C. Vanegas, M. Bhargava, N. Mechulan, P. Hendershot, H. Yamaguchi, C. Gomes, E.S. McLamore, A comparative study of graphene-hydrogel hybrid bionanocomposites for biosensing, *Analyst.* 140: 1466–1476 (2015). https://doi.org/10.1039/c4an01788a.

[80] M. Stoytcheva, R. Zlatev, G. Montero, Z. Velkova, V. Gochev, Bi-enzyme Electrochemical Sensor for Selective Determination of Organophosphorus Pesticides with Phenolic Leaving Groups, *Electroanalysis*. 29: 2526–2532 (2017). https://doi.org/10.1002/elan.201700380.

[81] M. Senel, M. Dervisevic, L. Esser, E. Dervisevic, J. Dyson, C.D. Easton, V.J. Cadarso, H.

Voelcker, Enhanced electrochemical sensing performance by In Situ electrocopolymerization of pyrrole and thiophene-grafted chitosan, *Int. J. Biol. Macromol.* 143: 582-593 (2020). https://doi.org/10.1016/j.ijbiomac.2019.12.013.

[82] S. Liu, L. Wang, W. Lian, H. Liu, C.Z. Li, Logic gate system with three outputs and three inputs based on switchable electrocatalysis of glucose by glucose oxidase entrapped in chitosan films, *Chem. - An Asian J.* 10: 225–230 (2015). https://doi.org/10.1002/asia.201402927.

[83] B.N. Aini, S. Siddiquee, K. Ampon, K.F. Rodrigues, Development of glucose biosensor based on ZnO nanoparticles film and glucose oxidase-immobilized eggshell membrane, *Sens. BIO-SENSING Res.* 4: 46–56 (2015). https://doi.org/10.1016/j.sbsr.2015.03.004.

[84] W. Dong, K. Wang, Y. Chen, W. Li, Y. Ye, S. Jin, Construction and Characterization of a Chitosan-Immobilized-Enzyme and β -Cyclodextrin-Included-Ferrocene-Based Electrochemical Biosensor for H_2O_2 Detection, *Materials.* 10(8): 868 (2017). https://doi.org/10.3390/ma10080868

[85] G. Huanan, G. Dezhuang, S. Yan, H. Bolin, Z. Na, Biosensor composed of integrated glucose oxidase with liposome microreactors / chitosan nanocomposite for amperometric glucose sensing, *Colloids Surfaces A.* 574: 260–267 (2019). https://doi.org/10.1016/j.colsurfa.2019.04.076.

[86] J. Lu, D. Cui, H. Li, Y. Zhang, S. Liu, Electrochimica Acta Cytochrome P450 bienzymes assembled on Au / chitosan/reduced graphene oxide nanosheets for electrochemically-driven drug cascade metabolism, *Electrochim. Acta.* 165: 36–44(2015). https://doi.org/10.1016/j.electacta.2015.02.183

[87] R.K. Satvekar, S.S. Rohiwal, A.P. Tiwari, A. V. Raut, B.M. Tiwale, S.H. Pawar, Sol-gel derived silica/chitosan/Fe_3O_4 nanocomposite for direct electrochemistry and hydrogen peroxide biosensing, *Mater. Res. Express.* 2: 015402 (2015). https://doi.org/10.1088/2053-1591/2/1/015402.

[88] D.F. Báez, S. Bollo, A comparative study of electrochemical performances of carbon nanomaterial-modified electrodes for DNA detection. Nanotubes or graphene ?, *J. Solid State Electrochem.* 1–6 (2015). https://doi.org/10.1007/s10008-015-2997-2.

[89] Z. Wang, J. Xia, D. Song, F. Zhang, M. Yang, R. Gui, L. Xia, S. Bi, Y. Xia, Lable-free quadruple signal amplification strategy for sensitive electrochemical p53 gene biosensing, *Biosens. Bioelectron.* 77: 157–163 (2016). https://doi.org/10.1016/j.bios.2015.09.011.

[90] R. Sorouri, H. Bagheri, A. Afkhami, J. Salimian, Fabrication of a Novel Highly Sensitive and Selective Immunosensor for Botulinum Neurotoxin Serotype A Based on an Effective Platform of Electrosynthesized, *Sensors.* 17(5): 1074 (2017). https://doi.org/10.3390/s17051074.

[91] S. Zhang, L. Zang, X. Zhang, H. Dai, G. Xu, Q. Zhang, C. Yang, Y. Lin, Signal-on

electrochemiluminescent immunosensor based on poly (amidoamine) dendrimer functionalized carbon nanodots amplification for ultrasensitive detection of α -fetoprotein, *Electrochim. Acta.* 196: 67-74 (2016). https://doi.org/10.1016/j.electacta.2016.02.162.

[92] W.J. Cheong, S.H. Yang, F. Ali, Molecular imprinted polymers for separation science: A review of reviews, *J. Sep. Sci.* 36: 609–628 (2013). https://doi.org/10.1002/jssc.201200784.

[93] A. McCluskey, C.I. Holdsworth, M.C. Bowyer, Molecularly imprinted polymers (MIPs): Sensing, an explosive new opportunity?, *Org. Biomol. Chem.* 5: 3233–3244 (2007). https://doi.org/10.1039/b708660a.

[94] A. Lamaoui, A.A. Lahcen, J.J. García-Guzmán, J.M. Palacios-Santander, L. Cubillana-Aguilera, A. Amine, Study of solvent effect on the synthesis of magnetic molecularly imprinted polymers based on ultrasound probe: Application for sulfonamide detection, *Ultrason. Sonochem.* 58: 104670 (2019). https://doi.org/10.1016/j.ultsonch.2019.104670.

[95] C. Salvo-Comino, I. Rassas, S. Minot, F. Bessueille, M. Arab, V. Chevallier, M.L. Rodriguez-Mendez, A. Errachid, N. Jaffrezic-Renault, Voltammetric sensor based on molecularly imprinted chitosan-carbon nanotubes decorated with gold nanoparticles nanocomposite deposited on boron-doped diamond electrodes for catechol detection, *Materials (Basel).* 13: 1–11 (2020). https://doi.org/10.3390/ma13030688.

[96] T. Gan, Z. Lv, Y. Sun, Z. Shi, J. Sun, A. Zhao, Highly sensitive and molecular selective electrochemical sensing of 6-benzylaminopurine with multiwall carbon nanotube@SnS2-assisted signal amplification, *J. Appl. Electrochem.* 46: 389–401 (2016). https://doi.org/10.1007/s10800-016-0923-7.

[97] B.L. Lu Lin, Hui-Ting Lian, Xiang-Ying Sun, Yaming Yu, L-dopa electrochemical sensor based on graphene doped molecularly imprinted chitosan film, *Anal. Methods*. 1387–1394 (2015). https://doi.org/10.1039/c4ay02524e.

[98] J. Srivastava, N. Gupta, A. Kushwaha, S. Umrao, A. Srivastava, M. Singh, Highly sensitive and selective estimation of aspartame by chitosan nanoparticles–graphene nanocomposite tailored EQCM-MIP sensor, *Polym. Bull.* 76:4431–49(2019). https://doi.org/10.1007/s00289-018-2597-2

[99] P. Wei, Z. Zhu, R. Song, Z. Li, C. Chen, An ion-imprinted sensor based on chitosan-graphene oxide composite polymer modified glassy carbon electrode for environmental sensing application, *Electrochim. Acta.* 317: 93–101 (2019). https://doi.org/10.1016/j.electacta.2019.05.136.

[100] H. Chakroun Galai, P. Namour, A. Bonhomme, F. Bessueille, S. Besbes Hentati, N. Jaffrezic-Renault, Elaboration of an Imprinted Polymer Film Based on Chitosan Electrodeposition for the Voltammetric Detection of BPA, *J. Electrochem. Soc.* 167: 027507 (2020).

https://doi.org/10.1149/1945-7111/ab6283.

[101] C. Salvo-Comino, I. Rassas, S. Minot, F. Bessueille, M.L. Rodriguez-Mendez, A. Errachid, N. Jaffrezic-Renault, Voltammetric sensor based on electrodeposited molecularly imprinted chitosan film on BDD electrodes for catechol detection in buffer and in wine samples, *Mater. Sci. Eng. C.* 110: 110667 (2020). https://doi.org/10.1016/j.msec.2020.110667.

[102] L. Chen, H.T. Lian, X.Y. Sun, B. Liu, Sensitive detection of L-5-hydroxytryptophan based on molecularly imprinted polymers with graphene amplification, *Anal. Biochem.* 526: 58–65 (2017). https://doi.org/10.1016/j.ab.2017.03.017.

[103] L. Wang, H. Yang, L. Xu, C. Peng, Y. Song, A novel popamine-imprinted chitosan/CuCo2O4@carbon/three-dimensional macroporous carbon integrated electrode, *J. Alloys Compd.* 817: 152771 (2020). https://doi.org/10.1016/j.jallcom.2019.152771.

[104] B. Liu, H. Lian, L. Chen, X. Wei, X. Sun, Differential potential ratiometric sensing platform for enantiorecognition of chiral drugs, *Anal. Biochem.* 574: 39–45 (2019). https://doi.org/10.1016/j.ab.2019.03.015.

[105] W.R. Zhao, T.F. Kang, L.P. Lu, F.X. Shen, S.Y. Cheng, A novel electrochemical sensor based on gold nanoparticles and molecularly imprinted polymer with binary functional monomers for sensitive detection of bisphenol A, *Journal of Electroanalytical Chemistry*, 786: 102-111 (2017). https://doi.org/10.1016/j.jelechem.2017.01.003.

[106] S. Wu, X. Dai, T. Cheng, S. Li, Highly sensitive and selective ion-imprinted polymers based on one-step electrodeposition of chitosan-graphene nanocomposites for the determination of Cr(VI), *Carbohydr. Polym.* 195: 199–206 (2018). https://doi.org/10.1016/j.carbpol.2018.04.077.

[107] F. Zouaoui, S. Bourouina-Bacha, M. Bourouina, I. Abroa-Nemeir, H. Ben Halima, J. Gallardo-Gonzalez, N. El Alami El Hassani, A. Alcacer, J. Bausells, N. Jaffrezic-Renault, N. Zine, A. Errachid, Electrochemical impedance spectroscopy determination of glyphosate using a molecularly imprinted chitosan, *Sensors Actuators, B Chem.* 309: 1–7 (2020). https://doi.org/10.1016/j.snb.2020.127753.

[108] Y. Wu, P. Deng, Y. Tian, Z. Ding, G. Li, J. Liu, Z. Zuberi, Q. He, Rapid recognition and determination of tryptophan by carbon nanotubes and molecularly imprinted polymer-modified glassy carbon electrode, *Bioelectrochemistry.* 131: 107393 (2020). https://doi.org/10.1016/j.bioelechem.2019.107393.