Conductive Hydrogel-Based Electrochemical Sensor: A Soft Platform for Capturing Analyte

Li Fu 1,*, Aimin Yu 2 and Guosong Lai 3

1 Key Laboratory of Novel Materials for Sensor of Zhejiang Province, College of Materials and Environmental Engineering, Hangzhou Dianzi University, Hangzhou 310018, China
2 Department of Chemistry and Biotechnology, Faculty of Science, Engineering and Technology, Swinburne University of Technology, Hawthorn, VIC 3122, Australia; aiminyu@swin.edu.au
3 Hubei Key Laboratory of Pollutant Analysis & Reuse Technology, College of Chemistry and Chemical Engineering, Hubei Normal University, Huangshi 435002, China; gslai@hbnu.edu.cn
* Correspondence: fuli@hdu.edu.cn

Abstract: Electrode modifications for electrochemical sensors attract a lot of attention every year. Among them, hydrogels are a relatively special class of electrode modifier. Since hydrogels often contain polymers, even though they are conductive polymers, they are not ideal electrode modifiers because of their poor conductivity. However, the micro-aqueous environment and the three-dimensional structure of hydrogels are an excellent platform for immobilizing bioactive molecules and maintaining their activity. This gives the hydrogel-modified electrochemical sensor the potential to perform specific recognition. At the same time, the rapid development of nanomaterials also makes the composite hydrogel have good electrical conductivity. This has led many scientists to become interested in hydrogel-based electrochemical sensors. In this review, we summarize the development process of hydrogel-based electrochemical sensors, starting from 2000. Hydrogel-based electrochemical sensors were initially used only as a carrier for biomolecules, mostly for loading enzymes and for specific recognition. With the widespread use of noble metal nanoparticles and carbon materials, hydrogels can now be used to prepare enzyme-free sensors. Although there are some sporadic studies on the use of hydrogels for practical applications, the vast majority of reports are still limited to the detection of common model molecules, such as glucose and H2O2. In the review, we classify hydrogels according to their different conducting strategies, and present the current status of the application of different hydrogels in electrochemical sensors. We also summarize the advantages and shortcomings of hydrogel-based electrochemical sensors. In addition, future prospects regarding hydrogel for electrochemical sensor use have been provided at the end.

Keywords: conductive hydrogels; electrochemistry; quantitative analysis; chemometrics; nanocomposites; analytical chemistry; food analysis; drug testing

1. Introduction

Hydrogel is a soft material with a three-dimensional network structure formed by cross-linking hydrophilic macromolecules through covalent, hydrogen or ligand bonds. Due to its unique porous structure and good flexibility, hydrogel shows excellent application prospects in flexible wearable electronics, batteries, and biosensors [1–7]. These applications require hydrogels with good electrical conductivity. Therefore, conductive hydrogels have been widely studied as an important member of the smart hydrogel family [8–10]. Conductive hydrogels, as a new functional material, were first proposed by Guiseppe-Elie et al. [11] and Wallace et al. [12], as a mixture of hydrogels with conductive polymers. After more than a decade of development, conductive hydrogels can be fabricated with good electrical conductivity, stability, and mechanical properties, which makes them more valuable for practical applications.
As a special type of sensor used in modern analytical chemistry, an electrochemical sensor is one in which a specific sensing element reacts with a target analyte to produce a sensing signal. It converts this signal into a recognizable electrical signal that is proportional to the concentration of the target analyte, by means of a specific transducer, thus achieving a qualitative or quantitative analysis [13–17]. The electrochemical sensor is mainly composed of the following two parts: a solidified sensing element (recognition system) and a transducer (conversion system). Its basic structure and principle are shown in Figure 1.

![Figure 1. The structure and basic principle of electrochemical sensor.](image)

The earliest studies of electrochemical sensors began in the 1950s, with the monitoring of oxygen. In the 1960s, based on the previous research on oxygen electrodes, new enzyme electrodes and ion-selective electrodes were developed, among which the “enzyme electrode”, firstly constructed by Clark et al. in 1962, was an important milestone [18]. They attached the dialysis membrane with fixed glucose oxidase to the oxygen electrode and then combined it with electrochemical technology. Using the chemical principle that glucose oxidase catalyzes the process of glucose oxidation by depleting dissolved oxygen, they monitored the concentration of glucose in solution by measuring the change in dissolved oxygen concentration in the solution. However, the enzyme electrodes constructed in this way could not be reused and therefore were not yet ready for practical use. It was not until 1967 that Updike and Hicks made a breakthrough on the issue of reproducibility [19]. They developed the world’s first glucose electrochemical sensor by using the new method of immobilizing glucose oxygenase on the surface of an oxygen electrode. Since then, electrochemical sensors have been widely used in the fields of pharmaceutical analysis and environmental monitoring, which is opening up a new era of electrochemical sensor research. After the 1970s, with the increasing amount of research, the existing electrochemical sensor technology is no longer sufficient for more precise scientific research. Therefore, the use of functional changes in the surface structure of the sensitive elements in electrochemical sensors, to control the surface electrochemical processes, can bring electrochemical sensor technology to a more precise level of differentiation [20–27].

As the basic theoretical research progressed, breakthroughs in the commercialization of electrochemical sensors were gradually achieved. The first commercialization of sensors was achieved in 1975, with the introduction of an enzyme electrode-based glucose detector by YSI Corporation (Yellow Springs, OH, USA). In 1984, Cass et al. [28] published the first paper on the development of an enzyme electrode method for media. This paper led directly to the successful development of the MediSene enzyme electrode sensor in 1987. Over the next few decades, new types of electrochemical sensors were gradually commercialized and became widely used in real-life applications. For example, a portable home blood glucose meter can measure the glucose concentration in blood samples at any time and is available in the market for only a few hundred China Yuan(CNY).
Generally speaking, nanomaterials are most commonly used for the modification of sensor electrodes [29–32]. Nanomaterials exhibit some properties that are unique from microscopic particles and macroscopic objects because of their particle size up to the nanoscale and the special effects of their large surfaces; for example, surface effect, volume effect, quantum size effect, and macroscopic quantum tunneling effect. These properties are incomparable to other conventional materials, and thus nanomaterials are widely used in electroanalysis [33]. By modifying the electrode with the above nanomaterials, the electrode will have a large specific surface area and more active sites on the surface, which can effectively accelerate the electron transfer rate and increase the rate of electrochemical reactions [34].

One- and two-dimensional nanomaterials were often used as modifiers [35]. However, designing three-dimensional gel structures allows for more rational control of chemical and physical properties [36]. In general, composite hydrogels are cross-linked polymer networks that are swollen with water at the nanoscale. These polymer networks often have unique mechanical, optical, thermal, magnetic and electrical response properties. In particular, the hydrogel itself can form a microenvironment that can confine the analyte. This is a strategy that allows the sensor to detect with high sensitivity [4,8]. Hydrogel is also an excellent material for the assembly of molecularly imprinted sensors because it provides a miniature aqueous environment and is able to maintain biological activity [37]. Therefore, in recent years, a number of works have also focused on the use of hydrogels for the preparation of electrochemical sensors.

This review focuses on the use of hydrogels as a sensing platform for electrochemical analysis applications. Since electrochemical sensors use electrical signals, hydrogels need to be conductive in some way. This review divides hydrogels into four sections according to their conductivity strategies. First, we briefly described the principles of each conductive strategy with some representative preparation methods. Then, we listed the applications of such hydrogels in electrochemical analysis. We compared the detection performance of the same detection substance by different electrochemical sensors. Based on the performance, we discussed their advantages and limitations. In addition, we highlighted their potential for future development in this field.

2. Polyelectrolyte Conductive Hydrogel

Polyelectrolyte conductive hydrogels are generally hydrogels formed by chemical or physical cross-linking polymerization of hydrophilic monomers containing ionic groups. The ionic groups in these hydrogels are the key to the high electrical conductivity of the hydrogels.

Wang et al. [38] prepared poly(2-propylene-amine-2-methylpropanesulfonate) (PAMPS) hydrogels with high ionic conductivity by the solid solution method (Figure 2A). They also investigated the effect of the content of the cross-linking agent sodium montmorillonite (NaMMT) on the ionic conductivity and tensile strength of the hydrogels. The results showed that the ionic conductivity and tensile strength of the hydrogels were good at the mass fraction of Na-MMT of 5–7%, where the ionic conductivity was $5.91 \times 10^{-2}$ S/cm and the tensile strength was 8.9 MPa. Later, they prepared polyelectrolyte hydrogels with high ionic conductivity and mechanical properties through a semi-interpenetrating polymer network (s-IPN) composed of PAMPS, polyvinyl alcohol (PVA), and Na-MMT [39]. In this work, they investigated the effect of the content of Na-MMT and PVA on the ionic conductivity and tensile strength of this hydrogel. The results showed that when the ratio of PVA/AMPS was 0.8:1 and the MMT was 7 wt.%, the ionic conductivity and tensile strength reached $6.4 \times 10^{-2}$ S/cm and 24.3 MPa, respectively. Diao et al. [40] prepared polyelectrolyte conducting hydrogels with PVA as the first network, and acrylamide copolymer with sulfobetaine methacrylate (P(AMco-SBMA)) as the second network, using a simple two-step copolymerization freeze–thaw method (Figure 2B). The effect on the ionic conductivity of this hydrogel was investigated by varying the content of amphoteric SBMA monomer. The results showed that the conductivity of the hydrogels increased from 0.33 to 2.89 S/m with the addition of 5 wt.% of SBMA, compared to no SBMA. The conductivity
of the hydrogel was increased from 0.33 to 2.89 S/m with the addition of 5 wt.% SBMA, and reached a maximum of 7.49 S/m with 15 wt.% SBMA. The highest conductivity of 7.49 S/m was achieved at 15 wt.% of SBMA. However, since their works were not focused on electrochemical sensors, the need for an interfacial response of electrochemical sensors was not taken into account in the design of the hydrogel. Therefore, although these works greatly enhance the conductivity of the electrodes, it does not mean that these hydrogels can be directly used for the assembly of electrochemical sensors.

This class of hydrogels is often used as a carrier for enzyme or probe loading, to achieve specific recognition in electrochemical analysis; for example, aldehyde oxidoreductase, isolated from *Escherichia coli*, can be loaded in poly(vinyl)imidazole hydrogels after using poly (ethylene glycol) diglycidyl ether as a crosslinker [41]. N,N-dimethylethanol moieties can be used for loading pyrroloquinoline quinone-dependent glucose dehydrogenase, flavin adenine dinucleotide-dependent glucose dehydrogenase, and FAD-dependent dehydrogenase [42]. The polyelectrolyte conductive hydrogel has also been used in the immobilization of acetylcholinesterase [43,44], horseradish peroxidase [45,46], glucose oxidase [47,48], lactate oxidase [49], alkaline phosphatase [50], horse heart cytochrome c [51,52], *Saccharomyces cerevisiae* cytochrome c peroxidase [53], organoselenium [53], and deoxyribonucleic acid (DNA) [54]. There are exceptions, such as Cizek et al. [55], who directly modified screen-printed electrodes with agar hydrogels and then used them for TNT detection. Liu et al. [56] reported the use of aldehyde-agarose hydrogel films for the detection of the promyelocytic leukemia/etnoic acid receptor alpha (PML/RARα) fusion gene in acute promyelocytic leukemia (APL). The diol group in the hydrogel was converted to an aldehyde group by a reaction with NaIO₄. With methylene blue as an electrochemical indicator, this sensor allows the highly sensitive detection of the PML/RARα fusion gene in APL. Since specific recognition is the underlying methodology of these sensors, hydrogels do not require particularly superior electrical properties.

Ionic liquids have also been used in the synthesis of hydrogels [57]; for example, Wang et al. [38] reported a sensor based on the photochemistry of polymerized ionic liquids. After polymerization, the ionic liquid can be used as a molecular imprinting platform for protein detection. Of course, the ionic liquid itself cannot provide an obvious signal. Therefore, AuNPs and ZnCdHgSe quantum dots (QDs) have also been used for
the assembly of the molecularly imprinted platform. AuNPs and ZnCdHgSe QDs were not used to enhance the conductivity of the hydrogel, but to provide a signal for detection. They investigated how well this sensor could detect human epididymis protein 4 (HE4). Under optimal conditions, a linear detection range of 25 pg/mL to 4.0 ng/mL, with a limit of detection of 15.4 pg/mL, could be achieved. Hydrogel is an excellent material for the assembly of molecularly imprinted sensors because it provides a miniature aqueous environment and is able to maintain biological activity. Except HE4, hemoglobin [59] has also been detected using this strategy. It is worth noting, however, that in our opinion ionic liquids are not a suitable candidate for promotion as electrochemical sensors, due to their cost.

The polymerization can also be conducted by means of electropolymerization. For example, chitosan can be deposited on the electrode surface by electrodeposition with negative potential, and a hydrogel can be formed [60]. If AgNPs were added during the polymerization process, they can be embedded at the same time. The prepared hydrogel can be used for the electrochemical detection of trichloroacetic acid. Electrochemical polymerization has the problem of certain reproducibility. Electrochemical polymerization is not a homogeneous reaction, so the electrode type, parameters during polymerization, and even the temperature and purity of the monomer during polymerization can affect the performance of the final sensor. Therefore, hydrogels synthesized using electrochemical polymerization require special attention for quality control.

3. Acid-Doped Conductive Hydrogel

In general, the conductive property of hydrogels can be improved by doping them with appropriate amounts of acid. As the dopant changes, the conductive property of the hydrogel also changes.

Li et al. [61] prepared poly(aniline)(PANI)/poly(propylene amide) hydrogels by in situ polymerization. The effect of two different acids (hydrochloric acid and p-toluenesulfonic acid), doped with PANI, on the hydrogel properties was investigated. The results showed that the high doping amount of hydrochloric acid and the low doping amount of p-toluenesulfonic acid helped to improve the electrical conductivity. The best electrical conductivity of the hydrogel was achieved when the ratio of hydrochloric acid to benzene was 10:3, and the ratio of p-toluenesulfonic acid to aniline was 1:2. Similarly, Prabhakar et al. [62] synthesized conductive polyacrylamide (PAM)/PANI hydrogels by interfacial polymerization, using PANI-impregnated PAM as the conductive material (Figure 3). Sulfuric acid (H$_2$SO$_4$), hydrochloric acid (HCl), and perchloric acid (HClO$_4$) were used as the different acidic dopants. This work explored the effect of dopant ions on the mechanical and conductive properties of the hydrogel. The conductivity of the different acid-doped hydrogels is as follows: H$_2$SO$_4$ > HCl > HClO$_4$. The H$_2$SO$_4$-doped PAM/PANI hydrogels showed the highest electrical conductivity and the best mechanical toughness compared with other dopants. In another work, they prepared PANI-impregnated acrylate conductive hydrogels using aqueous solution polymerization of interpenetrating polymer networks [63]. This work explored the effect of hydrochloric acid, nitric acid, and sulfuric acid dopants on the conductive properties of the hydrogels. The results showed that among the three inorganic acid dopants, sulfuric acid-doped hydrogels had the highest electrical conductivity, while nitric acid had the lowest. Since sulfuric acid is strongly acidic, the hydrochloric acid-doped hydrogel, with a good electrical conductivity of about 3.71 mS/cm, was prepared under optimized conditions. However, acids can affect not only the conductivity of hydrogels, but also the electrochemical behavior. The electrochemical behavior of molecules is often strongly related to the pH environment. Therefore, it is a challenge to try to strike a balance between detection performance and hydrogel conductivity.
Phytic acid (PA) is an exception that is used to form hydrogels, and is consequently used for sensing purposes [64,65]. A highly sensitive electrochemical detector for copper ions can be prepared by multiple interactions of dopamine (DA), acrylamide (AAM), and PA, under mild environmental conditions [64]. In this case, AAM is polymerized by free radicals, DA undergoes a multicoupling reaction, and PA crosslinks polydopamine (PDA) and PAM by hydrogen bonding. This hydrogel not only exhibits the highly sensitive detection of copper ions, but also acts as an adsorbent to remove copper ions from aqueous solutions. The change in boronic acid groups in hydrogels can also affect the capacitance of hydrogels [66]. Therefore, when glucose and boronic acid groups interact, the capacitance of the hydrogel changes in a positive correlation. This change can be used for the highly sensitive detection of glucose.

4. Inorganic Material Filled Conductive Hydrogel

Some inorganic particles have good electrical conductivity, such as metal nanoparticles, metal oxide nanoparticles, and some non-metal nanoparticles. The doping of inorganic materials with hydrogels gives the inorganic materials unique flexibility and ductility. On the other hand, this combination can also lead to enhanced mechanical properties of the hydrogel, overcoming the defect of coagulation and fragility; For example, Bao et al. [67] obtained an oligomeric suspension with a small relative molecular weight by mixing and reacting the supramolecular organic polymer Empol 1016 (Kerr, Italy) with diethylenetriamine. Then, micronickel with nanoscale surface characteristics was dispersed in this oligomeric matrix, which can effectively avoid its agglomeration phenomenon. The oxide layer on the surface of nanoparticles provides a large number of hydrogen bonds, which facilitates their binding to supramolecular organic polymers. The above two factors allow inorganic micronickel to be uniformly dispersed in supramolecular oligomers. This suspension is then reacted with uric acid under a strictly controlled reaction temperature and time, to convert only the primary amine to the urea group. The inorganic doped conductive hydrogel can be produced after mold shaping. The conductivity of the hydrogel was tested by a four-probe tester up to 40 S/cm. The mechanical tensile stress test showed that the addition of micronickel also greatly enhanced its mechanical properties. In addition, the presence of a large number of supramolecular hydrogen bonding forces in the hydrogel gives an excellent self-healing performance.
Alam et al. [68] prepared novel graphene/polyacrylic acid (PA) composite conductive hydrogels. They dissolved graphene in an aqueous solution of acrylic acid monomer, using N,N-methylenebisacrylamide and potassium persulfate as the crosslinker and initiator, respectively. They investigated the changes in the mechanical and conductive properties of this hydrogel after the addition of graphene. It was shown that the compressive strength increased from 0.37 to 6.9 Mpa with the addition of 1 vol% graphene, an increase of 1764%. The Young’s modulus increased from 1.64 to 19.03 Mpa. The electrical conductivity of the composite hydrogel was $1.56 \times 10^{-5}$ S/m. More similarly, Chen et al. [69] prepared PAM/CNF/CNT composite conductive hydrogels by the in situ polymerization of carbon nanotubes (CNTs) and cellulose nanofibers (CNFs) mixed in a solution of acrylamide (AM) (Figure 4). Among them, CNTs were used as conductive fillers. CNFs were used as reinforcing fillers for PAM and dispersing agents for CNTs. In this work, the effects of the additives CNT and CNF on the electrical conductivity and mechanical properties of the hydrogel were investigated. In addition, the results showed that the conductivity of the PAM/CNF/CNT hydrogel was $8.5 \times 10^{-4}$ S/cm after adding 1 wt.% CNF and 1 wt.% CNT to the PAM. At the same time, its tensile strength at break was three times higher than that of the pure PAM hydrogel, and the conductivity was twice as high as that of the PAM/CNT hydrogel.

Our previous works proposed an electrochemical sensor cross-linked with metal ions [70–72]. Zinc and silver ions can be used as cross-linking ions to enable the formation of hydrogels from chitosan and sodium alginate. The key point for the formation of these hydrogels is a suitable pH environment. A weakly acidic environment is required to enable the formation of such hydrogels. This hydrogel can easily depolymerize and release metal ions when subjected to oxidizing agents. Therefore, we used this hydrogel as an electrochemical sensing platform to measure the antioxidant activity. As shown in Figure 5A, the presence of antioxidants prevents the attack of hydrogen peroxide on chitosan and mitigates the polymerization process. The electrochemical reduction signal of metal ions, compared to the presence of antioxidants, can be used to assess the antioxidant activity of the analyte. In addition to our use of this technique for the antioxidant assessment of bee products [72,73], some other works have extended this technique for more sensitive sensing [74–76].
addition to the Ag⁺ and Zn²⁺ that we used, additional K⁺ was successfully used as a cross-linking agent. As shown in Figure 5B, K⁺ can facilitate the cation-templated assembly of the guanosine-quartet motif [77]. These guanosine-quartet motifs, stacked together with hemin, can form enzyme-like nanohydrogels. However, this hydrogel requires additional electrodeposition of PANI on the surface if it is to be used as an electrochemical sensor, due to its poor electrical conductivity. A similar work has been reported by Zhou et al. [78] for the detection of extracellular H₂O₂.

Carbon-based material is one of the most widely used inorganic materials for electrochemical sensing. Carbon materials are often used for electrode modifications because of their good electrical conductivity. More importantly, they do not participate in electrochemical reactions in a wide electrochemical window. In the preparation of hydrogels, many studies have incorporated carbon materials to improve the electrical conductivity and for electrochemical analysis; for example, graphite was added to chitin to prepare a hydrogel, and was used for the electrochemical determination of hydroquinone, catechol, resorcinol [79], and myoglobin [80]. The rise in carbon nanomaterials has led to their widespread incorporation into hydrogels, to provide excellent electrical conductivity. CNT are among the very important materials; for example, the deposition of poly(vinylimidazole) complexed with Os(4,4’-dimethylbpy)2Cl₂(PVI-dmeOs₂), on the surface of multi-walled carbon nanotubes (MWCNT), leads to a hydrogel [81]. This hydrogel has good electrical conductivity, and can respond quickly to glucose oxidase and lactate oxidase. Derkus et al. [82] investigated the immobilization effect of CNT-based hydrogels on proteins. A higher immobilization efficiency was obtained with the addition of CNT, due to their electrical conductivity and surface enhancement, especially in the gelatin-chitosan hydrogel.

**Figure 5.** (A) CV scans of 2.5 Mm zinc ions in (a) an acetic acid-sodium hydroxide solution, (b) the chitosan hydrogel, (c) the chitosan hydrogel with 0.01 Mm H₂O₂, and (d) the chitosan hydrogel with 0.03 Mm H₂O₂ [70]. (B) Schematic diagram of forming enzyme-like nanofibrous hydrogel from guanosine and B(OH)₃ via formation of K⁺-templated G4 quartet [77]. (C) Formation of CNT-based hydrogel [82]. (D) Formation of 3D graphene—AuNPs hydrogel [83]. Copyright: Elsevier B.V. and John Wiley and Sons.
After its discovery, graphene has become a new star among carbon materials. The addition of graphene to hydrogels, instead of carbon nanotubes, has become a hot research topic. The lamellar structure of graphene also makes it easier for the hydrogel to maintain a stable 3D structure; for example, Zhu et al. [83] reported a 3D graphene hydrogel. The raw material of this hydrogel only requires graphene oxide. The inorganic filler is gold nanoparticles. This hydrogel can be used for the simultaneous detection of ascorbic acid (AA), dopamine (DA), and uric acid (UA) (Figure 5C). Burrs et al. [84] systematically investigated the graphene-based hydrogel system. They prepared four graphene-based hydrogels for the immobilization of alcohol oxidase. The four hydrogels were chitosan, poly-N-isopropylacrylamide (PNIPAAM), silk fibroin, and cellulose nanocrystal (CNC) hydrogels. They used alcohol oxidase catalyzing the production of hydrogen peroxide from methanol as a model to investigate the sensing performance. It was found that these sensors were not very significantly superior in terms of detection performance, when compared to the other reports. However, all the materials of these hydrogel-based sensors are non-toxic. The sensors also do not require a mediator/adjuvant. Moreover, the shelf life and response time of these sensors were significantly improved compared to other reports. Table 1 summarizes inorganic material-filled hydrogel for electrochemical sensing. Notably, graphene is often used in the preparation of hydrogels, with its derivatives, such as graphene oxide and reduced graphene oxide. In hydrogel synthesis, the properties of graphene are difficult to control, due to the difference in the degree of reduction. Therefore, this leads to the fact that graphene-based hydrogels can have quality control challenges. This will need to be addressed in the future, with a more standard synthesis process.

Table 1. Summary of inorganic material-filled hydrogel for electrochemical sensing.

| Raw Material          | Inorganic Filler | Analyte                     | Reference |
|-----------------------|------------------|-----------------------------|-----------|
| Chitosan              | Zn$^{2+}$        | Antioxidant activity        | [70]      |
| Chitosan              | Ag$^+$           | Antioxidant activity        | [71]      |
| 1,3,5-benzenetricarboxylic acid | Fe$^{2+}$    | Neuron-specific enolase     | [85]      |
| Guanosine, B(OH)$_3$  | K$^+$            | Glucose                     | [77]      |
| Chitin                | Graphite         | Hydroquinone, catechol, resorcinol | [79] |
| Chitosan              | Au NPs           | Glucose                     | [86]      |
| Chitosan              | Laponite         | 1-lactate                   | [87]      |
| Graphene oxide        | Au NPs           | AA, DA, UA                  | [83]      |
| agarose                | Au NPs           | Carbamate                   | [88]      |
| PA, PANI, lutetium phthalocyanine | Graphene oxide | Glucose                     | [89]      |
| PVI-dmeOs             | MWCNT            | Glucose oxidase, lactate oxidase | [81] |
| PAM                   | Pyrolytic graphite | Myoglobin                   | [80]      |
| Poly(acrylic acid-co-itaconic acid) | MWCNT | Cd$^{2+}$                   | [90]      |
| Chitosan              | Graphene         | H$_2$O$_2$                  | [84]      |
| Chitosan              | Au NPs           | H$_2$O$_2$                  | [91]      |
| PNIPAAM               | Graphene         | H$_2$O$_2$                  | [84]      |
| Silk fibroin          | Graphene         | H$_2$O$_2$                  | [84]      |
| CNC                   | Graphene         | H$_2$O$_2$                  | [84]      |
| Graphene oxide        | PdCu NPs         | Glucose                     | [92]      |
| Nitrogen-doped activated graphene | Au NPs | Hydroquinone, o-dihydroxybenzene | [93] |
| Ferrocene (Fe) modified amino acid | Phenylalanine | Prostate specific antigen    | [94]      |
| Graphene oxide        | Au NPs           | Indole-3-acetic acid, salicylic acid | [95] |
| Pyrrole               | Graphene         | Ammonia gas                 | [96]      |
| Graphene oxide        | Au NPs           | Nitric oxide                | [97]      |
| β-cyclodextrin, chitosan | Au NCs        | H$_2$O$_2$                  | [98]      |
| Graphene              | Prussian blue    | H$_2$O$_2$                  | [99]      |
| Graphene oxide        | Co$_3$O$_4$      | Glucose                     | [100]     |
| Graphene oxide        | NiCo$_2$O$_4$    | H$_2$O$_2$                  | [101]     |
| Graphene oxide        | ZnO              | H$_2$O$_2$, glucose         | [102]     |
| Graphene oxide        | Au, Pt, Pd and Cu | UA                         | [103]     |
| Graphene oxide        | Pyrrole, Co$^{2+}$ | Catechol, hydroquinone      | [104]     |
5. Conductive Polymer-Based Conductive Hydrogel

Prior to the discovery of polyacetylene's high electrical conductivity, organic polymers were thought to be insulating materials. The discovery of the electrical conductivity of polyacetylene opened the research door for optoelectronics research [105]. It has demonstrated many applications in areas such as supercapacitors, chemical and biological sensors, lithium batteries, and memory materials. On one hand, conductive polymers have properties that are similar to plastics, such as a low mass density and high flexibility. At the same time, they have properties that are similar to those of metals, such as high electrical conductivity. The combination of these two properties makes them valuable in a variety of applications. In 1980, scientists reported higher-density polyacetylene [106], and then organometallics were used as catalysts to decompose cyclooctatetraene, to produce easily soluble polyacetylene [107]. More types of conductive polymers have been prepared. In particular, new types of conductive complexes have been synthesized using the original conductive polymers doped with other conductive or non-conductive components. Such polymers are synergistically enhanced with each other, in terms of conductivity, and also have the properties of another counterpart, such as catalytic, stability and oxidation resistance.

Conductive polymer-based conductive hydrogels are mixtures of polymers or the polymerization of conductive polymers doped in a three-dimensional network of hydrogels, which means that they not only have high electrical conductivity, but also have excellent properties of hydrogels. The commonly used conductive polymers are PANI and polyphenylene (PPPy); Hu et al. [108] prepared polyvinyl alcohol/glycerol/polyaniline (PGA) conductive hydrogels by organically combining PANI, PVA, and glycerol. This work discussed the effects of polyvinyl alcohol, glycerol, and benzylamine/phytic acid solution concentrations on the mechanical, electrical, and freezing resistance of PGA gels. The results show that the conductive PGA hydrogels have good electrical conductivity (0.335 S/m), fracture stress (472%), and fracture stress (94 KPa). In addition, the PGA conductive gel maintained good electrical conductivity (0.32 S/m) and mechanical properties at −20 °C. Shi et al. [109] synthesized a micro- and nano-structured conductive PPy hydrogel using the organic/water biphasic interfacial polymerization method. This method modulates the mechanical and electrochemical properties of this hydrogel by controlling the ratio of phytate-to-pyrrole monomers during the synthesis process. The doping of PPy with the azo dye molecule tartrazine also allows the formation of a 3D network of hydrogels (Figure 6) [110]. The good conductivity of this hydrogel can be used directly for the electrochemical detection of AA, DA, and UA. Table 2 summarizes the conductive polymer-based hydrogel for electrochemical sensing.
Table 2. Summary of conductive polymer-based hydrogel for electrochemical sensing.

| Conductive Polymer | Other Material | Analyte             | Reference |
|--------------------|----------------|---------------------|-----------|
| PPy                | Collagen       | Glucose             | [111]     |
| PANI               | PtNPs          | Glucose             | [112]     |
| PPy                | Tartrazine     | AA, DA, UA         | [110]     |
| PANI               | PAM            | UA                  | [113]     |
| PPy                | PANI           | Glucose, urea       | [114]     |
| PPy                | -              | Concentration of the electrolyte | [115] |
| PPy                | p(2-hydroxyethyl methacrylate) | Glucose | [116] |
| PANI               | Graphene       | Pb^{2+}             | [117]     |

6. Other Hydrogels

There are also some hydrogels that cannot be included in the above classifications. These types of hydrogels are used for electrochemical sensing, but do not have any components that are used to enhance their electrical properties. These hydrogels are often only used in a platform for the immobilization of biomolecules such as enzymes; for example, a peptide hydrogel composed of N-fluorenylmethoxycarbonyl-diphenylalanine was proposed for the immobilization of horseradish peroxidase enzyme [118]. This peptide hydrogel not only provides a good immobilization platform, but also maintains the enzyme activity well, and can therefore be used to detect H_2O_2 in cells. PAM and chitosan have been used for preparing hydrogel to immobilize hemoglobin. This hydrogel has also been used for detecting H_2O_2 [119].

Changes in the electrical signal upon hydrogel film cleavage can also be used for sensing analysis. Contacting a hydrogel film containing a specific cleavage site for matrix metalloproteinase-9 (MMP-9) with oxidized dextran can lead to cleavage of the hydrogel [120]. The impedance changes during this cleavage process. This change can be used for...
the detection of MMP-9, a peripheral biomarker of neuroinflammation in multiple sclerosis (MS). This sensor has the potential to replace MRI as diagnostic evidence for MS. A DNA hydrogel can be synthesized by target cycling and a hybridization chain reaction (HCR), with Hg$^{2+}$-induced activation of Mg$^{2+}$-specific DNAase [121]. This hydrogel can be used for the highly sensitive detection of Hg$^{2+}$, by alteration of the electrochemical impedance. Similar DNA hydrogels can also be formed by cross-linking ferrocene-labeled recognition probes with DNA grafted on a polyacrylamide backbone [122]. DNA hydrogels have also been used for the electrochemical detection of the platelet-derived growth factor [123] and H$_2$O$_2$ [124]. An introduction and the trends in biosensing using DNA hydrogels can be found in Li et al. article [125].

7. Conclusions and Perspectives

This review summarizes the progress of hydrogels in electrochemical sensors. Overall, hydrogels are starting to be increasingly used for the preparation of electrochemical sensors and to explore their applications in bioanalysis. Hydrogels can provide a matrix to preserve the biological activity of biomolecules, due to the special micro-water environment. At the same time, hydrogels have a very large specific surface area because of their 3D structure. Therefore, hydrogels were mainly used as a platform for the immobilization of biomolecules in early studies. These hydrogels, loaded with biomolecules, can be used for specific recognition of the analyte. However, hydrogels are mainly composed of polymers, and even though some polymers are conductive, they are still not very ideal materials for assembling electrochemical sensors. Therefore, how to improve the electrical properties of hydrogels has captured the attention of scientists in the last decade. Due to the rapid development of nanomaterials, especially carbon nanomaterials and noble metal nanomaterials, many composite hydrogels already possess excellent electrical properties. However, improving the electrochemical properties of a hydrogel does not mean that it will work well as an electrochemical sensor. Electrochemical sensors require that the analyte is involved in an electrochemical reaction at the electrode interface. Therefore, it is equally important to improve the ability of the hydrogel interface to capture the analyte. Therefore, future research needs to focus on the mechanism of interaction between different molecules and hydrogels. In addition to simple adsorption, the use of functional groups in the hydrogel composition, to grab the analytes, is also effective.

Moreover, some carbon materials, such as graphene, can be assembled into hydrogels with lamellar monomers, which ensures their usefulness in electrochemical sensors. We believe that a nanosheet of other 2D materials can also be assembled into hydrogels as monomers in the future. As different 2D materials are discovered and investigated, non-organic hydrogels will become increasingly available. This can fundamentally overcome the electrical defects of polymer-based hydrogels. At the same time, the addition of other effective components to the new hydrogels can achieve the same wide range of applications as conventional electrochemical sensors. However, due to their inherent hydrophobicity, carbon materials are often used in the synthesis of hydrogels with their oxidized derivatives. The surface oxidation of carbon materials significantly affects their electrical properties, so hydrogels are often subject to reduction after synthesis. However, the degree of reduction is a challenge for these carbon materials during their application. How to precisely control the degree of reduction in hydrogels of carbon materials is an issue to be overcome in the future.

For the time being, hydrogel electrochemical sensors do not have a performance advantage over other conventional electrochemical sensors. In many cases, their detection performance is not as good as that of the more methodologically advanced solid-state electrochemical sensors. However, hydrogel sensors have certain advantages in terms of stability, reproducibility, and shelf life. Therefore, good performance will be expected if very popular sensing methodologies, such as immunosensing and DNA sensing, are applied to hydrogel electrochemical sensors. Studies now use those molecules that are more commonly tested, such as blood glucose and H$_2$O$_2$. These molecules are already available with proven electrochemical sensors for highly sensitive detection. The special
microenvironment of hydrogels can ensure a more stable performance of these sensing strategies. Therefore, how to take advantage of the hydrogel’s strengths, to address some of the detection needs that require a specific microenvironment, is also the future focus of this field.

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