



Application of Graphene and Chitosan in Water Splitting/Catalysis

Nimra Iqbal¹, Shaukat Ali^{1,2}, Asif Hanif Chaudhry³, Nosheen Sial⁴, Syed Asim Abbas Zaidi¹,
Waqar Ahmad Murtaza^{1†} and Shumaila Shabbir¹

¹Department of Chemistry, Superior University, Lahore, Pakistan

²Building Research Station, Communication and Works Department, Lahore, Pakistan

³Geological Survey of Pakistan, Lahore, Pakistan

⁴Government Graduate College for Women, Gulberg, Lahore, Pakistan

†Corresponding author: Waqar Ahmad Murtaza; mallikwaqarmurtaza@gmail.com

Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 29-04-2024

Revised: 12-06-2024

Accepted: 18-06-2024

Key Words:

Electrical double layer
Carboxy graphene
Chemical doping
Engineering of graphene
Protein desorption
Catalysis

ABSTRACT

This study aims to explore the applications of graphene and chitosan in water splitting and catalysis, focusing on their unique properties and synergistic effects. A comprehensive review of the literature was conducted to examine their roles in photocatalytic activity and environmental remediation. Graphene, known for its high surface area and conductivity, was analyzed for its ability to enhance charge separation and light harvesting through doping and hybridization with metal nanoparticles. Similarly, chitosan's biopolymeric nature and strong affinity for transition metals were evaluated for their utility in enzymatic and catalytic applications. Results indicate that graphene's photocatalytic performance can be significantly improved through doping and functionalization, while chitosan proves effective in wastewater treatment and as a polymeric support for catalysts. The study concludes that the combined use of graphene and chitosan offers promising potential for advancing sustainable energy solutions and environmental technologies.

INTRODUCTION

For the production of solar fuel cells, photocatalysis is one of the most important techniques used. Due to its remarkable properties and production potential, graphene materials are considered attractive for water splitting. To improve charge separation and visible light harvesting, graphene-based materials are commonly used as additive agents to enhance the photocatalytic activity of graphene. For further improvement of graphene's photocatalytic activity, small amounts of metal and metal oxide nanoparticles are introduced onto the surface of graphene. Additionally, two special features, crystal alignment, and solid grafting, are recognized in graphene-based materials to enhance water splitting. Because of its large surface area and high sorption capacity, graphene is projected as an excellent catalyst for catalysis applications. The most significant applications of graphene in catalysis include the use of graphene-based materials as electrocatalysts and doped graphene as catalysts. Chitosan is an optically active polymer recognized for its strong affinity for transition metals. Polymers can be utilized as heterogeneous catalysts in forms such as gels, beads, and colloids. Chitosan is particularly suitable for enzymatic catalysis in various catalytic applications.

CHITOSAN

Historical Background

In 1179, Hachet decalcified the shells of crabs, lobsters, prawns, and crayfish using mineral acid and observed that they produced moderately energetic, soft, and plastic material of a yellowish color, resembling cartilage but retaining their original shape. This was the first mention of calcified chitin in invertebrates.

The discovery of chitin's characteristics began in 1811 when Braconnot identified chitin in fungi. Later, in 1823, another scientist, Odier, extracted a horn-like material from cockchafer elytra using potassium hydroxide. Braconnot initially named the material "chitin," a term later adopted and popularized by Odier. In 1824, the nitrogenous nature of chitin was revealed by the researchers referred to as "the children." By 1894, Hoppe-Seyler introduced the term "chitosan," with Lederhose identifying its first derivative, Glykosamin, in 1876 (Crini 2019).

Properties of Chitosan

Typically, naturally occurring polysaccharides such as

pectin, dextrin, agar, agarose, and cellulose are acidic, but chitosan is an extremely basic polysaccharide (Jayakumar et al. 2010). Chitosan exhibits superior properties such as viscosity, solubility, metal chelation, optical activity, polyelectronic behavior, the ability to form films, and structural characteristics. It is considered an important candidate for tissue engineering, particularly for regenerating connective tissue (Shukla et al. 2013). In bone formation, chitosan plays a significant role in the creation of osteoblasts. It also displays antifungal, hemostatic, antitumor, and anti-cholesteremic properties (Franco & Peter 2010; Dutta et al. 2004).

The heteropolymer of chitosan contains glucosamine and acetyl-glucosamine units. Properties such as acid-base behavior and solubility can be controlled by the relative portion of chitosan, which regulates the degree of acylation (Deutsche Chemische Gesellschaft 1888; Sorlier et al. 2001). The carbohydrate backbone of chitosan is similar to cellulose. In chitosan, the $-OH$ group on the second carbon atom is replaced by an $-NH_2$ group or an acetyl group. The copolymer consists of two repeating units: N-acetyl-2-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose. These repeating units are linked by (1–4) glycosidic bonds, which give chitosan a rigid crystalline structure due to intermolecular and intramolecular bonding (Roberts & Robert 1992, Dash et al. 2011).

Chitosan contains one $-NH_2$ group and two $-OH$ groups on each glycosidic unit, which indicates that it is a polycationic polymer (Agrawal et al. 2010). Due to these two reactive groups, chitosan exhibits unique chemical and biological properties. Chitosan is an ideal candidate for biofabrication because its active amino group is reactive and provides a platform for side group attachment under various

reaction conditions (Yi et al. 2005). By altering its biological and physical properties, chitosan can provide elasticity and enhance functionality. The pKa value of chitosan depends on the ionic strength, diacylation, and charge neutralization of the $-NH_2$ group. The pKa value typically lies between 6.3 and 6.7 when the degree of acylation is no more than 50% (Suh et al. 2000).

Applications of Chitosan

Most industries discharge large amounts of wastewater containing inorganic and organic contaminants such as dyes, toxicants, suspended solids, and pesticides. This wastewater creates serious environmental issues and poses a threat to water quality, even when discharged into rivers and lakes. The treatment of wastewater can be made possible with the help of the nontoxic and biodegradable biopolymer chitosan, as it carries a partial positive charge, making it an effective polycationic coagulating agent in water treatment. Chitosan is helpful because it attracts heavy metal ions for separation from wastewater and also binds to all groups of transition metal ions at very low concentrations. Due to the presence of the amino group on chitosan, it serves as a chelation agent for transition metal ions.

Advantages and Disadvantages of Chitosan

Advantages of chitosan: Chitosan, which is derived from chitin, has wide biomedical and pharmaceutical advantages due to its widespread availability, biocompatibility, biodegradability, non-toxicity, and high drug-loading capacity. Chitosan is very important for drug delivery applications in the treatment of various diseases. It is also useful for improving patient compliance in infectious disease drug therapy (Rajitha et al. 2016).

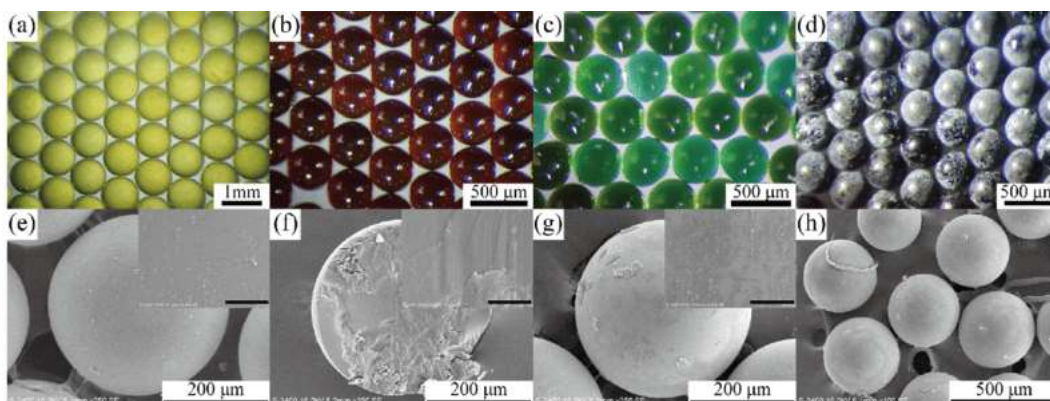


Fig. 1: Optical micrographs of (a) chitosan emulsion templates with crosslinking time 30 min and (b) PEI-CS biosorbents; Optical micrographs of (c) CS biosorbents and (d) PEI-CS bio sorbents after adsorption equilibrium; The SEM micrographs of (e) the surface and (f) inner structures of a CS biosorbent, (g) the surface of a PEI-CS biosorbent and (h) a certain amount of PEI-CS biosorbents. The scale bar of insert images in (e)–(g) is 20 μ m (Rodrigues et al. 2012).

Techniques like emulsion cross-linking, which involves the emulsification of an aqueous solution of chitosan in oil, in the presence of an appropriate emulsifier, followed by its stabilization using crosslinkers to form nanoparticles enhance its application in forming nanoparticles for targeted drug delivery (Venkatesan et al. 2013, Rodrigues et al. 2012). Optical and SEM micrographs, shown in Fig. 1, highlight the structure and efficiency of chitosan emulsion templates and biosorbents, highlighting their potential in biomedical and environmental applications.

Another technique i.e. spray drying in which nanoparticle powder is obtained through a single-step process. The drug, dissolved in an aqueous solution of chitosan in acetic acid, is atomized with a crosslinker (Wang et al. 2011). This is also beneficial for the application in targeted drug delivery

Furthermore, chitosan demonstrates adhesive properties, with its amino and carboxyl groups forming hydrogen bonds with glycoproteins in mucus. This interaction improves drug bioavailability and ensures sustained drug release (Jin & Hu 2008).

Additionally, chitosan exhibits antitumor effects by interfering with cell metabolism and act on tumor cells to inhibit cell growth or induce apoptosis. It plays a vital role in enhancing the body's immune function as an antitumor agent (Cao & Zhou 2005, Maeda & Kimura 2004). Studies have shown that low molecular weight chitosan and chito-oligosaccharides can obstruct tumor growth in S180-bearing mice. Torzsas et al. (1996) demonstrated that a diet containing chitosan could reduce the formation of precancerous lesions in colon cancer.

Disadvantages of chitosan: Despite its advantages, chitosan

faces limitations such as poor solubility in neutral and alkaline solutions, scalability challenges, and variability in its properties depending on its source and preparation method. Additionally, its performance can be affected by environmental conditions, requiring further research to optimize its stability and functionality in diverse applications.

GRAPHENE

Graphene has captured the interest of the scientific community due to its distinctive properties, which are not found in traditional materials. It is a two-dimensional material capable of maintaining a tensile elastic strain exceeding 20%, providing an exciting opportunity to tune its strain properties. This has opened a new field known as straintronics.

Known for its exceptional mechanical strength, high electrical and thermal conductivity, and large surface area, graphene stands as a revolutionary material in various fields. Its zero-band-gap nature and ability to undergo functionalization further enhance its applicability across a wide range of disciplines, from electronics to energy systems. One of the most significant advancements in graphene research is its application in photocatalysis and water splitting. To address the limitations posed by its zero-band-gap property, graphene is often doped with heteroatoms or combined with metal and metal oxide nanoparticles, effectively enhancing its catalytic activity. This tailored approach allows graphene to serve as an effective catalyst for processes requiring high electron mobility and stability.

Recent advancements in characterizing and assembling graphene have made some approaches scalable, while others remain at the laboratory scale (Ghany et al. 2017).

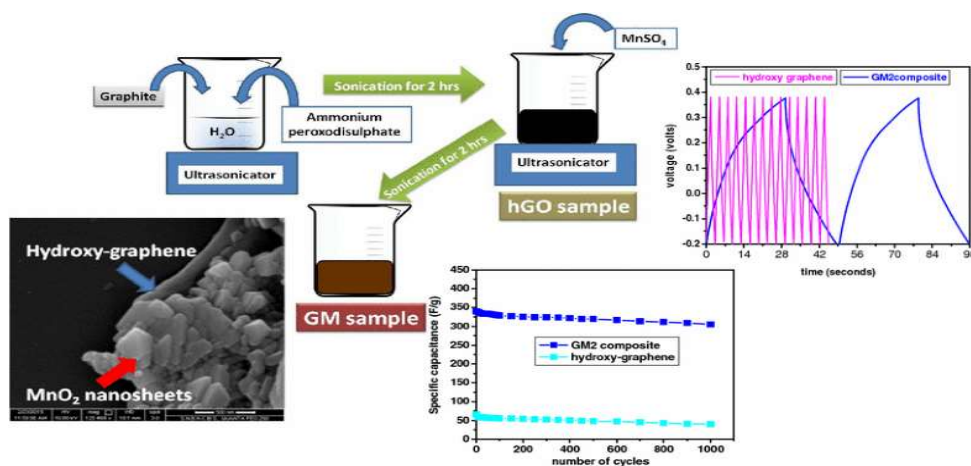


Fig. 2: Synthesis of functionalization hydroxy graphene (Brodie 1859).

Properties of Graphene

Graphene exhibits a wide range of unique properties that make it highly versatile for scientific and industrial applications. Its derivatives further enhance its functionality by introducing specific chemical and structural modifications. Below are the key properties of graphene:

Hydroxy graphene: Hydroxy graphene is synthesized through the hydroboration of graphene oxide, an addition reaction on carbon-carbon double bonds that results in an organoboron moiety. The preparation of graphol involves reacting fluorinated graphite or iodographene with sodium hydroxide under solvothermal conditions. This one-step process is simple and high-yielding, where hydrophilic hydroxyl substituents allow water molecules to penetrate the layers, leading to complex exfoliation.

Hydroxyl groups in graphene are critical for enhancing tribological properties by introducing hydrogen bonding within the layer lattice structure, improving inter-sheet shear resistance. Fig. 2 illustrates the synthesis of functionalized hydroxy graphene.

Carboxy graphene: For several applications, such as electrochemical sensing and transparent conductive films assembled with inorganics, the carboxylic acid functional group on the graphene structure is crucial. The carboxylic (COOH) functional groups are highly hydrophilic, significantly enhancing the stability of colloidal solutions and improving the dispersibility of graphene in water.

Carbon atoms located at the edges and defect sites of graphene are more reactive due to their higher volatility. These reactive sites are utilized to form covalent bonds with larger molecules, enabling control over graphene oxide (GO) separation and transport properties. This also

enhances its sorption capacity for heavy metals, making it valuable for environmental remediation applications.

Additionally, the presence of COOH groups on GO improves its mechanical properties. These groups contain electronegative oxygen atoms, which strongly influence the electronic structure of the material (Jeon et al. 2012).

Functionalization of graphene oxide (GO): Like other chemical systems, stoichiometric derivatives exhibit a high degree of functionalization. In 2D material research, there is significant interest in structures with mixed compositions. Graphene oxide (GO) contains a non-stoichiometric number of oxygenated groups and graphene derivatives, where the presence of carboxyl and hydroxyl groups is particularly important.

For transformation and device implementation, graphene can covalently interact with other elements, such as hydrogen (graphane), fluorine (fluorographene), and sulfur (thiographene) (Pumera & Sofer 2017).

Electrical double-layer: Using the theory of the electrical double layer, the surface charge of graphene oxide (GO) can be described as a flat slab with two faces. At high electrolyte concentrations, the pH conditions and the presence of electrolytes determine the surface charge density, interactions between GO sheets, and the potential.

GO can orient and migrate in the presence of a magnetic field. The interaction between GO and the magnetic field will vary depending on the size of the GO sheets. These factors are crucial in influencing the separation of GO during electromagnetic separation (Fig. 3).

Interaction: Graphene oxide (GO) can be described as a single-layer graphite sheet with various hydrophilic oxygenated functional groups. The ionizable groups are

ELECTRICAL DOUBLE LAYER

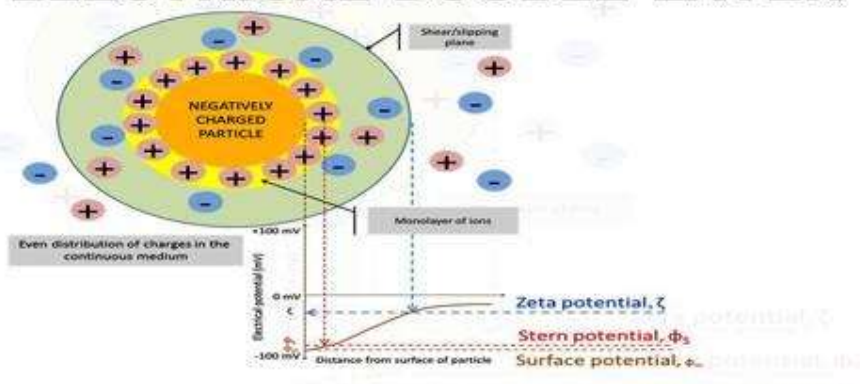


Fig. 3: Study of electrical double layer (Zhao et al. 2011).

located at the edges of the GO sheet, while the hydroxyl and epoxide groups are present on the basal planes.

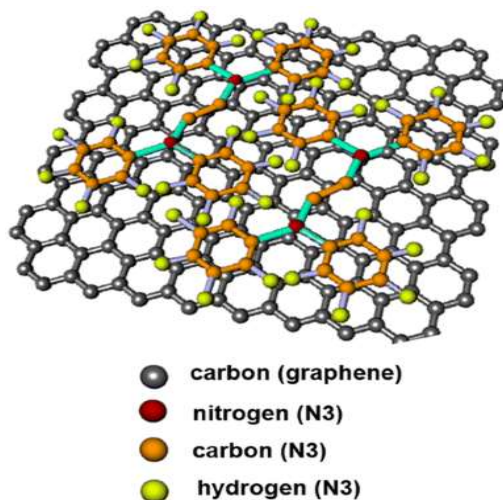
The ionized carboxyl and phenol groups generate electrostatic repulsion between the GO sheets, preventing aggregation in an aqueous medium. By reducing the cross-dimensional size of the GO sheet, the surface area-to-volume ratio increases. Due to the higher density of ionized groups, GO sheets exhibit enhanced solubility (Li & Muller 2008).

Stacking and structural variability: In graphene oxide (GO), the hydrogen, oxygen, and carbon atoms are organized into a monolayer with a thickness of approximately 1 nm, depending on the degree of hydration and the method used for its preparation, quantity, and thickness. The shape of GO particles can vary, appearing as flakes, quantum dots, sheets, ribbons, or plates, with dimensions ranging from nanometers (e.g., 4 nm) to millimeters (e.g., 3 mm).

GO membranes can be fabricated through the self-assembly of hydrosol by evaporation or by spin coating to create ultra-thin GO foils, a few nanometers thick, suitable for radio frequency resonators (Thebo et al. 2018).

Applications of Graphene

Graphene is a conductive material that can replace traditional electrode materials, such as indium tin oxide, in optical and electrical devices. It also possesses several advantageous properties, including high optical transparency, low sheet resistance, and excellent mechanical properties. The following applications of graphene include electrode materials in transistors, light-emitting diodes, solar cells, and flexible devices. Through synthesis, modification, and doping strategies, the performance of graphene can be further enhanced.



Optoelectronic properties: Graphene is a conductive material that can replace traditional electrode materials such as indium tin oxide in optical and electrical devices. Its high optical transparency, low sheet resistance, and excellent mechanical properties make it ideal for use in transistors, light-emitting diodes, solar cells, and flexible devices. By altering the thickness of graphene films, sheet resistance, and visible light transmission can be independently adjusted to desired values, enhancing performance in transparent electrodes.

Engineering of graphene: Graphene has become an attractive material for transparent conductive electrodes due to its low optical absorption and high conductivity. Graphene films with high conductivity and low optical loss can be engineered by applying various methods to achieve:

1. Development of graphene technology
2. Surface modification of graphene films
3. Doping of graphene films

Work function tuning: Recent investigational studies have revealed that work function engineering can be achieved by shifting the Fermi level of the graphene band structure through electric fields (Yu et al. 2009), dipole formation, and metal contacts (Giovannetti et al. 2018), in addition to doping. Controlling the graphene work function is essential for reducing the contact barrier in graphene electrode devices such as transistors, solar cells, and light-emitting diodes (Jo et al. 2010; Lee et al. 2011).

Chemical doping: Graphene, a two-dimensional material, consists of a single atomic layer of sp^2 carbon atoms. By shifting the Fermi level of the graphene band structure away from the Dirac point, the carrier concentration of the carbon

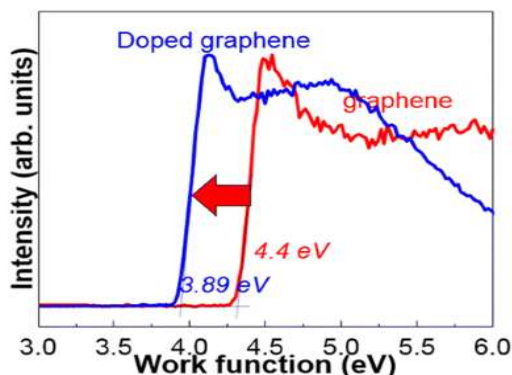


Fig. 4: Doping process in graphene (Wu et al. 2011).

layer is adjusted, which helps to improve the conductivity of the graphene films where the density is zero (Nistor et al. 2011). To modulate the conductive properties of graphene, the desired rigid band shift can be induced through chemical doping (Bae et al. 2010; Huh et al. 2011), electrostatic gating, metal contact, or dipole formation (Li et al. 2010; Huang et al. 2011). For example, the achievement of elements such as B or N (Wang et al. 2009) can be accomplished through hole (p) or electron (n) doping, which directly substitutes into the carbon lattice during layer growth to donate or remove electrons from the delocalized pz-band. Fig. 4 illustrates the doping process.

Advantages and Disadvantages of Graphene

Advantages: Graphene nanoplatelets (GNPs) are added to epoxy resins, improving properties such as mechanical, thermo-mechanical, thermal diffusivity, electrical conductivity, hydrophobic behavior, and barrier properties. The accumulation of GNPs leads to a significant stiffening of thermosetting resins, with notable increases in thermal diffusivity and electrical conductivity. These properties are proportional to each other—when thermal diffusivity increases, electrical conductivity also rises. By measuring the contact angle of a water drop, it is observed that GNP addition enhances the hydrophobic behavior, which is an advantage of graphene. However, excessive accumulation of GNPs reduces the diffusion coefficient and increases water absorption. The addition of carbon nanotubes improves thermal and mechanical properties while also increasing electrical conductivity. Low thermal interference resistance and two-dimensional properties make these types of graphene effective fillers for manufacturing composite materials, enhancing thermal conductivity (Chatterjee et al. 2012, Prolongo et al. 2013).

Graphene also plays a crucial role in photocatalytic applications. It is used as an additive to semiconductor materials, improving photocatalytic activity by enhancing charge separation and light harvesting. These features make graphene highly effective in water splitting and solar fuel cell production (Albero et al. 2019).

Mateo et al. (2016) focused on the development of renewable fuels from solar light, which is a major challenge in energy science today. Over the past decade, hydrogen and oxygen have been produced from solar light and water using various photocatalysts. However, photon-to-hydrogen mole conversion remains a real application of solar fuel cells. Facet-oriented gold nanoplates or multilayer graphene films, placed on quartz, serve as highly active photocatalysts for water splitting, converting sunlight into oxygen and hydrogen in the absence of a sacrificial electron donor, achieving

hydrogen production. Durable gold-graphene interaction occurs in the composite system, and photocatalytic activity arises from the preferential orientation. Additionally, it has been suggested that constructing three-dimensional permeable carbon foam, pretreated by glutaraldehyde cross-linking and decorated with discrete molybdenum carbide (CF-Glu-MO) or co-doped FeNi carbonate hydroxide (CF-Glu-CoFeNi), which is structurally derived from chitosan hydrogel, could further improve photocatalytic performance (Ding et al. 2021).

Disadvantages: Despite its advantages, excessive accumulation of graphene nanoplatelets can lead to reduced diffusion coefficients and increased water absorption, limiting their effectiveness in certain applications. The high production cost of graphene materials remains a significant challenge, restricting their scalability for industrial use. Furthermore, maintaining consistent photocatalytic performance over extended periods requires addressing issues such as material degradation and efficiency losses.

GRAPHENE AND CHITOSAN AS SURFACE ENHANCERS

Sulfonated Graphene Oxide and Nanohybrid Membranes

Sulfonated graphene oxide (SGO), sulfonic acid, and nanosheets are synthesized using a facile distillation, polymerization, and precipitation method. These components are then incorporated into a chitosan matrix to prepare nanohybrid membranes. The physiological and microstructural properties of these membranes are extensively studied.

Due to the strong electrostatic attraction between the SO_3H groups of SGO and the $-\text{NH}_2$ groups of chitosan, both GO-filled membranes and chitosan/SGO-filled membranes exhibit enhanced thermal and mechanical properties, which reduce the mobility of chitosan chains. This reduction in mobility decreases the swelling area of the SGO-filled membranes, thereby increasing their structural stability. Conductivity can be optimized by adjusting the content of chitosan and sulfonic acid groups. For example, incorporating 2% S4GO results in a 122% increase in hydrated conductivity and a 90% increase in anhydrous conductivity, compared to the control chitosan membrane. Other conduction properties show significant improvement in H_2 and O_2 cells, demonstrating that the nanohybrid membrane holds promise as a proton exchange membrane. Fig. 5 shows a polymer brush functionalized Janus graphene oxide/chitosan hybrid membranes.

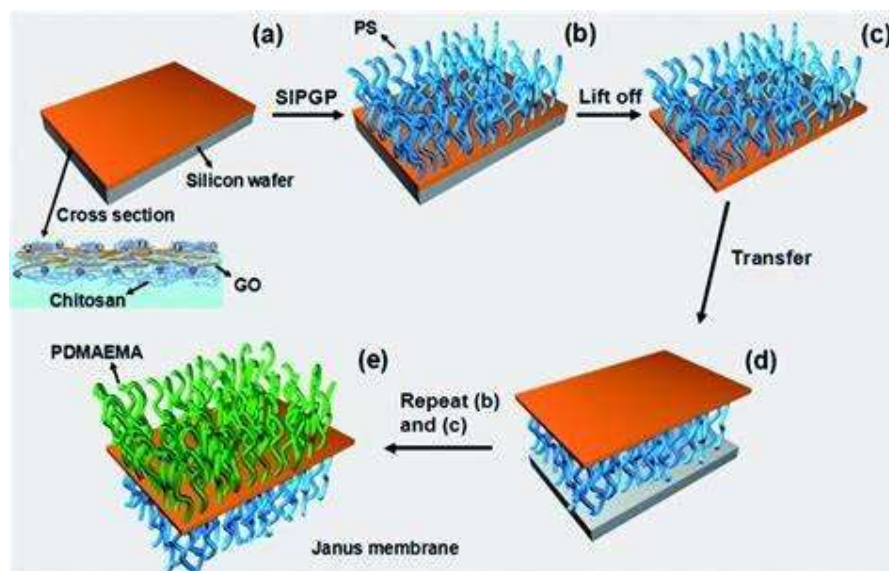


Fig. 5: Polymer brush functionalized Janus graphene oxide/chitosan hybrid membranes.

Application of Graphene in Water Splitting

Graphene-based materials as photocatalysts: Due to electronic band overlap, graphene is a zero-band-gap semiconductor. When electrons and holes recombine suddenly, the conductive properties of graphene block its application in photocatalysis. To address this, the band gap can be opened by doping with heteroatoms. In comparison to ideal graphene, graphene oxide (GO) and reduced graphene oxide (r-GO) both contain oxygen functionalities and act as semiconductor materials. Minor alterations to the oxygenated functional groups change the properties of graphene, converting the sp^2 carbon atoms into sp^3 . Due to low electron mobility, graphene oxide behaves as a p-type semiconductor. The band gap of graphene oxide ranges from 2.4 to 3.4 eV, depending on the oxidation level (Liu et al. 2014). The band gap increases with higher oxygen content. The valence band maximum (VBM) gradually shifts from the G π orbital to the oxygen 2p orbital, while the conduction band minimum (CBM) remains unchanged in the G π orbital (Ito et al. 2008).

Graphene for electrocatalytic water splitting: Graphene, with a single layer of carbon atoms arranged in a honeycomb structure, has various applications in water splitting. Ideal graphene has a zero-band-gap property with poor catalytic activity. However, graphene offers many benefits for electrocatalytic water splitting. When doped, graphene can act as an effective catalyst for water splitting. When graphene is hybridized with an electroactive component, the following benefits occur:

- The conductivity of the graphene hybrid increases, accelerating charge transfer kinetics.
- It improves the dispersion of the loaded catalyst, providing more catalytic sites.
- It prevents catalyst degradation, aggregation, and sintering under severe reaction conditions, thereby increasing the lifetime of the electrocatalyst.
- It regulates the electronic structure of the active center due to the combined interaction between graphene and transition metal catalysts (TM-Cs), improving their catalytic process.

Graphene hybrids for water splitting: Recent studies have shown that chemically doped graphene and graphene hybrids have superior catalytic activity and stability, making them well-suited for hydrogen evolution reactions (HER) and oxygen evolution reactions (OER). HER typically occurs in acidic electrolytes, while OER occurs in basic media. Both HER and OER are especially attractive for bifunctional catalysts (Sayama & Arakawa 1997).

From alkaline to neutral pH: Transition metal clusters (TMCs) have been primarily studied as active catalysts in several pH environments. They show excellent OER activity in alkaline media, which is useful for practical applications in alkaline water electrolyzers based on TMC bifunctional catalysts. In the OER catalyst family, Co-Pi/Co-Bi are the only potential catalysts that perform well in neutral media, but they are inert for HER. However, the development of a systematic bifunctional catalyst for overall water splitting in neutral media remains a significant challenge.

From monofunctional her/oer electrocatalysts to bifunctional catalysts for overall water splitting: Transition metal clusters (TMCs) possess bifunctional activity for both HER and OER. To improve the performance of TMC catalysts, graphene is considered a star material and is coupled with TMCs to create graphene hybrids. These hybrids act as bifunctional catalysts, providing superior performance for overall water splitting.

Application of Chitosan in Water Splitting

Enzymatic production of water-soluble chitosan: A chitinolytic enzyme complex produced by the gram-positive bacteria *Streptomyces kurssanovii* was immobilized on macroporous cross-linked chitin via physical adsorption. This biocatalyst was used to synthesize acid-free water-soluble chitosan with a molecular weight of 2-9 kDa through two-step hydrolysis. The first hydrolysis, at pH 4.6, produced chitosan with a solubility of 22-24 kDa in water, while the second hydrolysis at pH 6.2 resulted in acid-free, water-soluble chitosan (Gamzazade et al. 1985).

Immobilization of chitinolytic enzymes: The chitinolytic enzyme complex is produced by the Gram-positive bacterium *Streptomyces kurssanovii*, which is used for the enzymatic production of low-molecular-weight (LMW) chitosan (Ilyina et al. 1999). Unfortunately, the use of this chitosan for biomedical and food applications is restricted due to the pyrogenicity of the chitosan, which results from the presence of approximately 0.1% (w/w) of the protein enzyme complex.

Protein desorption: The interaction between the enzyme and the polymer matrix complex (PMC) under optimum conditions involves weak hydrogen bonds in the chitosan solution, which is exposed to the immobilized enzyme for hydrolysis. In the first 3 hours, a significant decrease in intrinsic viscosity occurs. After the next 24 hours, only small changes are observed, but these do not result in LMW chitosan. The final product, 24 kDa, has low solubility in acid-free water (Tikhonov et al. 1998).

Biocatalytic stability and PMC chitin regeneration: A two-step chitin hydrolysis method (3 hours at pH 4.6 and 0.5 hours at pH 6.2) was repeated 80 times, with complete recovery of the PMC chitin matrix, allowing further immobilization of chitinolytic enzymes (Miller 1959). After a 50% loss of its initial catalytic activity, the second-step biocatalyst was used. The *S. kurssanovii* enzyme complex is utilized in a large-scale process suitable for the production of acid-free, water-soluble LMW chitosan, which is physically adsorbed onto a stable, macroporous, cross-linked chitin matrix. This method allows the production of LMW chitosan oligosaccharides with minimal protein contamination.

Application of Graphene in Catalysis

Doped graphene in catalysis: In several reactions, graphene materials are doped with various heteroatoms, which are being explored as effective metal-free catalysts (Kong et al. 2014). Among these, nitrogen (N)-doped graphene has been widely studied. N-doping is frequently achieved by reacting graphene oxide (GO) with ammonia (Li et al. 2009), lithium nitride (Deng et al. 2014), aniline, or by CVD (Wei et al. 2009) and arc discharge methods (Li et al. 2010). Nitrogen-doped graphene has most of its applications in oxidation-reduction reactions related to fuel cells (Lee et al. 2010, Lin et al. 2013). N-graphene behaves as an excellent metal-free catalyst for oxidation-reduction reactions in alkaline fuel cells. Other catalytic applications of N-graphene include the reduction of nitro compounds (Kong et al. 2013, Chen et al. 2012), peroxides (Long et al. 2012), and the oxidation of glucose (Wang et al. 2010) and benzyl alcohol (Shao et al. 2010). Like N-graphene, sulfur-doped graphene is also used as a metal-free catalyst with high stability and selectivity in oxidation-reduction reactions. For hydrolysis reactions, sulfur-doped graphene exhibits good water tolerance and high reactivity. The catalytic applications of sulfated graphene include the dehydration of xylose and the esterification of acetic acid (Lam et al. 2012, Liu et al. 2012), among others.

Graphene in photocatalysis: In photocatalysis, graphene-based materials have significant applications, including reactions such as pollutant degradation, selective organic transformations, and water splitting for hydrogen energy production, with graphene being used as a photocatalyst (Zhang et al. 2012). The hybridization of graphene with various metal catalysts helps improve photocatalytic performance due to the extended light absorption range, high specific surface area, and excellent electron conductivity of graphene. By hybridizing graphene oxide with organic dyes or photocatalysts, photosensitization can be promoted through charge transfer across the graphene interface, producing a synergistic effect that enhances catalytic transformation.

Application of Chitosan in Catalysis

Chitosan-based Schiff Bases (CSBs): In heterogeneous catalysis, the role of CSBs as supports is crucial because they can attach catalytically active metal ions or metal nanoparticles, which provide effective reusability. These supports can be either organic polymers or inorganic materials. Polymers that are functionalized or have functional groups are preferred over other types of polymers, as their chemical interactions help prevent metal ion leaching from the support. Inorganic materials, such as silica, require very

strong reaction conditions to become functionalized, whereas chitosan contains inherent NH₂ groups that can easily form CSBs. In heterogeneous catalysis, chitosan proves to be an effective polymeric support due to its biodegradability. CSB catalysts are used in C-C coupling (Peng et al. 1998), oxidation reactions (Shen et al. 2017), reduction reactions (Gong et al. 2012), arylation, and cyclopropanation.

Enzymatic catalysis: The model for any chemical reaction aimed at achieving measurable yields under mild, energy-efficient, low-cost, and safe conditions, with minimal separation steps and without the production of toxic or harmful substances, is catalyzed by enzymes. Chitosan dissolved in dilute organic acid solutions readily precipitates, forming inclusion complexes. Enzymes can be chemically attached to chitosan through reactive groups (-OH and -NH₂). Chitosan is non-toxic, has high protein affinity, and is also inexpensive (Zhao et al. 2015).

Limitations

- Graphene and chitosan in water splitting, particularly in hydrogen production, face stability challenges such as scalability, cost-effectiveness, and durability.
- Graphene has a high production cost, and its catalytic activity decreases over time.
- Chitosan is renewable and biodegradable, but it suffers from stability issues under water-splitting conditions.
- Chitosan has partial durability and large-scale cost execution challenges.

FUTURE PERSPECTIVES

Graphene and chitosan show promise for future research and development across various fields, including advanced biomedicine, sustainable technology, energy revolution, smart materials, high-performance composites, nanotechnology, digital transformation, and environmental remediation. Overall, graphene and chitosan hold significant potential for addressing global challenges and driving innovation across numerous sectors in the years to come.

CONCLUSIONS

The review highlights that the photocatalytic activity of graphene can be enhanced for water splitting when graphene is doped with various metals such as H, N, P, B, and S, which opens the band gap of graphene-based materials. The photocatalytic activity of graphene can further be improved by adding small amounts of metal nanoparticles to graphene-based materials. Graphene, with its good chemical stability, can reduce catalyst bleaching, aggregation, and sintering

under reaction conditions. Graphene-based materials and hybrids are beneficial for overall water splitting. From the above discussion, it is clear that chitosan and its composite materials can be functionalized with considerable control. Chitosan is also useful for enzyme immobilization and enzymatic catalysis due to its low cost and non-toxic nature. Additionally, chitosan is employed as a biocatalyst for PMC chitin regeneration.

REFERENCES

- Agrawal, P., Strijkers, G.J. and Nicolay, K., 2010. Chitosan-based systems for molecular imaging. *Advanced Drug Delivery Reviews*, 62(1), pp.42-58.
- Albero, J., Mateo, D. and García, H., 2019. Graphene-based materials as efficient photocatalysts for water splitting. *Molecules*, 24(5), p.906.
- Bae, S., Kim, H., Lee, Y., Xu, X., Park, J.S., Zheng, Y., Balakrishnan, J., Lei, T., Ri Kim, H., Song, Y.I. and Kim, Y.J., 2010. Roll-to-roll production of 30-inch graphene films for transparent electrodes. *Nature Nanotechnology*, 5(8), pp.574-578.
- Brodie, B.C., 1859. XIII. On the atomic weight of graphite. *Philosophical Transactions of the Royal Society of London*, 149, pp.249-259.
- Cao, J. and Zhou, N.J., 2005. Progress in antitumor studies of chitosan. *Chinese Journal of Biochemical Pharmacology*, 26(2), p.127.
- Chatterjee, S., Wang, J.W., Kuo, W.S., Tai, N.H., Salzmann, C., Li, W.L., Hollertz, R., Nüesch, F.A. and Chu, B.T., 2012. Mechanical reinforcement and thermal conductivity in expanded graphene nanoplatelets reinforced epoxy composites. *Chemical Physics Letters*, 531, pp.6-10.
- Chen, T.W., Xu, J.Y., Sheng, Z.H., Wang, K., Wang, F.B., Liang, T.M. and Xia, X.H., 2012. Enhanced electrocatalytic activity of nitrogen-doped graphene for the reduction of nitro explosives. *Electrochemistry Communications*, 16(1), pp.30-33.
- Crini, G., 2019. Historical review on chitin and chitosan biopolymers. *Environmental Chemistry Letters*, 17(4), pp.1623-1643.
- Dash, M., Chiellini, F., Ottenbrite, R.M. and Chiellini, E., 2011. Chitosan—A versatile semi-synthetic polymer in biomedical applications. *Progress in Polymer Science*, 36(8), pp.981-1014.
- Deng, D., Pan, X., Yu, L., Cui, Y., Jiang, Y., Qi, J., Li, W.X., Fu, Q., Ma, X., Xue, Q. and Sun, G., 2011. Toward N-doped graphene via solvothermal synthesis. *Chemistry of Materials*, 23(5), pp.1188-1193.
- Deutsche Chemische Gesellschaft, 1888. *Berichte der Deutschen Chemischen Gesellschaft*. Berlin: Verlag Chemie.
- Ding, J., Zhong, L., Huang, Q., Guo, Y., Miao, T., Hu, Y., Qian, J. and Huang, S., 2021. Chitosan hydrogel derived carbon foam with typical transition-metal catalysts for efficient water splitting. *Carbon*, 177, pp.160-170.
- Dutta, P.K., Dutta, J. and Tripathi, V.S., 2004. Chitin and chitosan: Chemistry, properties and applications.
- Liu, F., Sun, J., Zhu, L., Meng, X., Qi, C. and Xiao, F.S., 2012. *Journal of Materials Chemistry*, 22, pp.5495-5502.
- Franco, T.T. and Peter, M.G., 2010. Advances in chitin science. *Santiago*, 6.
- Gamzazade, A.I., Šlimak, V.M., Skljarić, A.M., Štykova, E.V., Pavlova, S.S. and Rogožen, S.V., 1985. Investigation of the hydrodynamic properties of chitosan solutions. *Acta Polymerica*, 36(8), pp.420-424.
- Ghany, N.A., Elsherif, S.A. and Handal, H.T., 2017. Revolution of Graphene for different applications: State-of-the-art. *Surfaces and Interfaces*, 9, pp.93-106.
- Giovannetti, G.A., Khomyakov, P.A., Brocks, G., Karpan, V.V., van den Brink, J. and Kelly, P.J., 2008. Doping graphene with metal contacts. *Physical Review Letters*, 101(2), p.026803.

- Gong, S.W., He, H.F., Zhao, C.Q., Liu, L.J. and Cui, Q.X., 2012. Convenient deoxygenation of aromatic ketones by silica-supported chitosan Schiff base palladium catalyst. *Synthetic Communications*, 42(4), pp.574-581.
- Huang, J.H., Fang, J.H., Liu, C.C. and Chu, C.W., 2011. Effective work function modulation of graphene/carbon nanotube composite films as transparent cathodes for organic optoelectronics. *ACS Nano*, 5(8), pp.6262-6271.
- Huh, S., Park, J., Kim, K.S., Hong, B.H. and Kim, S.B., 2011. Selective n-type doping of graphene by photo-patterned gold nanoparticles. *ACS Nano*, 5(5), pp.3639-3644.
- Ilyina, A.V., Tatarinova, N.Y. and Varlamov, V.P., 1999. The preparation of low-molecular-weight chitosan using chitinolytic complex from *Streptomyces kurssanovii*. *Process Biochemistry*, 34(9), pp.875-878.
- Ito, J., Nakamura, J. and Natori, A., 2008. Semiconducting nature of the oxygen-adsorbed graphene sheet. *Journal of Applied Physics*, 103(11).
- Jayakumar, R., Menon, D., Manzoor, K., Nair, S.V. and Tamura, H., 2010. Biomedical applications of chitin and chitosan based nanomaterials - A short review. *Carbohydrate Polymers*, 82(2), pp.227-232.
- Jeon, I.Y., Shin, Y.R., Sohn, G.J., Choi, H.J., Bae, S.Y., Mahmood, J., Jung, S.M., Seo, J.M., Kim, M.J., Wook Chang, D. and Dai, L., 2012. Edge-carboxylated graphene nanosheets via ball milling. *Proceedings of the National Academy of Sciences*, 109(15), pp.5588-5593.
- Jin, H.Q. and Hu, Q.H., 2008. Characterization and application in bioadhesive drug delivery system of chitosan. *Centre South Pharm*, pp.324-327.
- Jo, G., Na, S.I., Oh, S.H., Lee, S., Kim, T.S., Wang, G., Choe, M., Park, W., Yoon, J., Kim, D.Y. and Kahng, Y.H., 2010. Tuning of a graphene-electrode work function to enhance the efficiency of organic bulk heterojunction photovoltaic cells with an inverted structure. *Applied Physics Letters*, 97(21).
- Kong, X.K., Chen, C.L. and Chen, Q.W., 2014. Doped graphene for metal-free catalysis. *Chemical Society Reviews*, 43(8), pp.2841-2857.
- Kong, X.K., Sun, Z.Y., Chen, M. and Chen, Q.W., 2013. Metal-free catalytic reduction of 4-nitrophenol to 4-aminophenol by N-doped graphene. *Energy & Environmental Science*, 6(11), pp.3260-3266.
- Lam, E., Chong, J.H., Majid, E., Liu, Y., Hrapovic, S., Leung, A.C. and Luong, J.H., 2012. Carbocatalytic dehydration of xylose to furfural in water. *Carbon*, 50(3), pp.1033-1043.
- Lee, K.R., Lee, K.U., Lee, J.W., Ahn, B.T. and Woo, S.I., 2010. Electrochemical oxygen reduction on nitrogen doped graphene sheets in acid media. *Electrochemistry Communications*, 12(8), pp.1052-1055.
- Lee, S., Jo, G., Kang, S.J., Wang, G., Choe, M., Park, W., Kim, D.Y., Kahng, Y.H. and Lee, T., 2011. Enhanced charge injection in pentacene field-effect transistors with graphene electrodes. *Advanced Materials*, 23(1), pp.100-105.
- Li, D., Muller, M.B., Gilje, S., Kaner, R.B. and Wallace, G.G., 2008. *Nature Nanotechnology*, pp.3-101.
- Li, N., Wang, Z., Zhao, K., Shi, Z., Gu, Z. and Xu, S., 2010. Large scale synthesis of N-doped multi-layered graphene sheets by simple arc-discharge method. *Carbon*, 48(1), pp.255-259.
- Li, X., Magnuson, C.W., Venugopal, A., An, J., Suk, J.W., Han, B., Borysiak, M., Cai, W., Velamakanni, A., Zhu, Y. and Fu, L., 2010. Graphene films with large domain size by a two-step chemical vapor deposition process. *Nano Letters*, 10(11), pp.4328-4334.
- Li, X., Wang, H., Robinson, J.T., Sanchez, H., Diankov, G. and Dai, H., 2009. Simultaneous nitrogen doping and reduction of graphene oxide. *Journal of the American Chemical Society*, 131(43), pp.15939-15944.
- Lin, Z., Waller, G.H., Liu, Y., Liu, M. and Wong, C.P., 2013. 3D Nitrogen-doped graphene prepared by pyrolysis of graphene oxide with polypyrrole for electrocatalysis of oxygen reduction reaction. *Nano Energy*, 2(2), pp.241-248.
- Liu, Y., Wang, J., Zhang, H., Ma, C., Liu, J., Cao, S. and Zhang, X., 2014. Enhancement of proton conductivity of chitosan membrane enabled by sulfonated graphene oxide under both hydrated and anhydrous conditions. *Journal of Power Sources*, 269, pp.898-911.
- Long, J., Xie, X., Xu, J., Gu, Q., Chen, L. and Wang, X., 2012. Nitrogen-doped graphene nanosheets as metal-free catalysts for aerobic selective oxidation of benzylic alcohols. *ACS Catalysis*, 2(4), pp.622-631.
- Maeda, Y. and Kimura, Y., 2004. Antitumor effects of various low-molecular-weight chitosans are due to increased natural killer activity of intestinal intraepithelial lymphocytes in sarcoma 180-bearing mice. *The Journal of Nutrition*, 134(4), pp.945-950.
- Mateo, D., Esteve-Adell, I., Albero, J., Royo, J.F.S., Primo, A. and Garcia, H., 2016. 111 oriented gold nanoplatelets on multilayer graphene as visible light photocatalyst for overall water splitting. *Nature communications*, 7(1), p.11819.
- Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, 31(3), pp.426-428.
- Nistor, R.A., News, D.M. and Martyna, G.J., 2011. The role of chemistry in graphene doping for carbon-based electronics. *ACS Nano*, 5(4), pp.3096-3103.
- Peng, C., Wang, Y., Tan, S. and Cheng, G., 1998. Preparation of chitosan derivatives. Synthesis of N-Schiff base type and N-secondary amino type chitosan-crown ethers. *Polymer Journal*, 30(10), pp.843-845.
- Prolongo, S.G., Jimenez-Suarez, A., Moriche, R. and Ureña, A., 2013. In situ processing of epoxy composites reinforced with graphene nanoplatelets. *Composites Science and Technology*, 86, pp.185-191.
- Pumera, M. and Sofer, Z., 2017. Towards stoichiometric analogues of graphene: graphane, fluorographene, graphol, graphene acid and others. *Chemical Society Reviews*, 46(15), pp.4450-4463.
- Rajitha, P., Gopinath, D., Biswas, R., Sabitha, M. and Jayakumar, R., 2016. Chitosan nanoparticles in drug therapy of infectious and inflammatory diseases. *Expert Opinion on Drug Delivery*, 13(8), pp.1177-1194.
- Roberts, G.A.F. and Robert, G.A.F. (Ed.), 1992. *Chitin Chemistry*. MacMillan, Houndmills, pp.1-53.
- Rodrigues, S., Dionísio, M., Remunan Lopez, C. and Grenha, A., 2012. Biocompatibility of chitosan carriers with application in drug delivery. *Journal of Functional Biomaterials*, 3(3), pp.615-641.
- Sayama, K. and Arakawa, H., 1997. Effect of carbonate salt addition on the photocatalytic decomposition of liquid water over Pt-TiO₂ catalyst. *Journal of the Chemical Society, Faraday Transactions*, 93(8), pp.1647-1654.
- Shao, Y., Zhang, S., Engelhard, M.H., Li, G., Shao, G., Wang, Y., Liu, J., Aksay, I.A. and Lin, Y., 2010. Nitrogen-doped graphene and its electrochemical applications. *Journal of Materials Chemistry*, 20(35), pp.7491-7496.
- Shen, C., Qiao, J., Zhao, L., Zheng, K., Jin, J. and Zhang, P., 2017. An efficient silica supported Chitosan@ vanadium catalyst for asymmetric sulfoxidation and its application in the synthesis of esomeprazole. *Catalysis Communications*, 92, pp.114-118.
- Shukla, S.K., Mishra, A.K., Arotiba, O.A. and Mamba, B.B., 2013. Chitosan-based nanomaterials: A state-of-the-art review. *International Journal of Biological Macromolecules*, 59, pp.46-58.
- Sorlier, P., Denuzière, A., Viton, C. and Domard, A., 2001. Relation between the degree of acetylation and the electrostatic properties of chitin and chitosan. *Biomacromolecules*, 2(3), pp.765-772.
- Suh, J.K. and Matthew, H.W., 2000. Application of chitosan-based polysaccharide biomaterials in cartilage tissue engineering: a review. *Biomaterials*, 21(24), pp.2589-2598.
- Thebo, K.H., Qian, X., Wei, Q., Zhang, Q., Cheng, H.M. and Ren, W., 2018. Reduced graphene oxide/metal oxide nanoparticles composite membranes for highly efficient molecular separation. *Journal of Materials Science & Technology*, 34(9), pp.1481-1486.
- Tikhonov, V.E., Radigina, L.A., Yamskov, I.A., Gulyaeva, N.D., Ilyina, A.V., Anisimova, M.V. and Varlamov, V.P., 1998. Affinity purification of major chitinases produced by *Streptomyces kurssanovii*. *Enzyme and Microbial Technology*, 22(2), pp.82-85.

- Torzsas, T.L., Kendall, C.W.C., Sugano, M., Iwamoto, Y. and Rao, A.V., 1996. The influence of high and low molecular weight chitosan on colonic cell proliferation and aberrant crypt foci development in CF1 mice. *Food and chemical toxicology*, 34(1), pp.73-77.
- Venkatesan, C., Vimal, S. and Hameed, A.S., 2013. Synthesis and characterization of chitosan triphosphate nanoparticles and its encapsulation efficiency containing Russell's viper snake venom. *Journal of Biochemical and Molecular Toxicology*, 27(8), pp.406-411.
- Wang, J.J., Zeng, Z.W., Xiao, R.Z., Xie, T., Zhou, G.L., Zhan, X.R. and Wang, S.L., 2011. Recent advances of chitosan nanoparticles as drug carriers. *International Journal of Nanomedicine*, pp.765-774.
- Wang, X., Li, X., Zhang, L., Yoon, Y., Weber, P.K., Wang, H., Guo, J. and Dai, H., 2009. N-doping of graphene through electrothermal reactions with ammonia. *Science*, 324(5928), pp.768-771.
- Wang, Y., Shao, Y., Matson, D.W., Li, J. and Lin, Y., 2010. Nitrogen-doped graphene and its application in electrochemical biosensing. *ACS Nano*, 4(4), pp.1790-1798.
- Wei, D., Liu, Y., Wang, Y., Zhang, H., Huang, L. and Yu, G., 2009. Synthesis of N-doped graphene by chemical vapor deposition and its electrical properties. *Nano Letters*, 9(5), pp.1752-1758.
- Wu, Z.S., Ren, W., Xu, L., Li, F. and Cheng, H.M., 2011. Doped graphene sheets as anode materials with superhigh rate and large capacity for lithium ion batteries. *ACS Nano*, 5(7), pp.5463-5471.
- Yi, H., Wu, L.Q., Bentley, W.E., Ghodssi, R., Rubloff, G.W., Culver, J.N. and Payne, G.F., 2005. Biofabrication with chitosan. *Biomacromolecules*, 6(6), pp.2881-2894.
- Yu, Y.J., Zhao, Y., Ryu, S., Brus, L.E., Kim, K.S. and Kim, P., 2009. Tuning the graphene work function by electric field effect. *Nano Letters*, 9(10), pp.3430-3434.
- Zhang, N., Zhang, Y. and Xu, Y.J., 2012. Recent progress on graphene-based photocatalysts: current status and future perspectives. *Nanoscale*, 4(19), pp.5792-5813.
- Zhao, J., Chen, G., Zhang, W., Li, P., Wang, L., Yue, Q., Wang, H., Dong, R., Yan, X. and Liu, J., 2011. High-resolution separation of graphene oxide by capillary electrophoresis. *Analytical Chemistry*, 83(23), pp.9100-9106.
- Zhao, J., Xie, Y., Guan, D., Hua, H., Zhong, R., Qin, Y., Fang, J., Liu, H. and Chen, J., 2015. BaFe₁₂O₁₉-chitosan Schiff-base Ag(I) complexes embedded in carbon nanotube networks for high-performance electromagnetic materials. *Scientific Reports*, 5(1), p.12544.