CHAPTER II

REVIEW OF LITERATURE

2.1 Chitin polymer

With the exception of cellulose, chitin is the most abundant natural polysaccharide on Earth. It is synthesized by different crustaceans, molluscs, marine diatoms, insects, algae, fungi, and yeasts. Offal's obtained during the processing of crab, lobster, shrimp, Antarctic krill, clams, and oysters consists in some cases of up to 75% shellfish total weight. At the present time only a small quantity of shell waste is utilized for animal feed or for chitin isolation. Thus, the processing of shellfish leads to environmental pollution. Recently, the commercial value of chitin has increased because of the beneficial properties of its soluble derivatives, which are suitable in chemistry, biotechnology, agriculture, food processing, cosmetics, veterinary, medicine, dentistry, environment protection, and paper or textile production.

The industrial production of chitin is limited by season of crustacean harvesting and a limited supply of the shell waste in some countries, and environmental pollution caused by alkali deproteinization producing waste liquid containing base, proteins, and protein degradation products. Because chitin and its soluble derivative chitosan are the principal components of the cell walls of several Zygomycetes, attention has been drawn to fungi for use as an alternative
resource of both polysaccharides available in a desirable amount using microorganism cultivation on an inexpensive media. In this review the data concerning the utilization of insect’s cuticles for are excluded. The resources of insect biomass are huge; however, the availability of this material for industrial processing is limited only to silkworm pupa (Bombyx mori) accumulated in a relatively small amount in the silk reeling industry.

Chitin is the primary structural component of the exoskeletons of crustaceans, molluscs, insects, some fungi and yeast. However, chitin is not present in higher plants and higher animals. The role played by chitin is similar to the roles played by cellulose in plants and collagen in higher animals. The annual biosynthesis of chitin has been estimated to 109 to 1011 tons. Chitin is widely distributed in nature; this is a renewable bioresource. The shell of selected crustacean was reported by Knorr [34] to consist of 30-40% protein, 30-50% calcium carbonate and calcium phosphate, and 20-30% chitin. The main commercial sources of chitin are the shellfish waste such as shrimps, lobster, crabs, squid pens, prawns and crawfish.

Structurally, chitin is a β-1-4, 2-acetamido-2-deoxy-D-glucopyranose sugar, where all residues contain N-acetyl glucosamine. Chitin has a molecular weight between 200 and 700K Daltons [35]. A major component in the shells of crustacean and mollusks, chitin is predominant in the backbones of squids and insect cuticles. Chitin is also found in fungi, algae, and protozoa. It is estimated that approximately 100 billion tons of chitin are synthesized per year, of that the
shell fish industry generates 150,000 tons of chitin/year. The structure of chitin is shown in Figure 2.1.

![Chemical structure of chitin polymer](image)

**Figure 2.1: Chemical structure of chitin polymer**

### 2.2 Types of chitin

There are two structures of chitin that can be formed that are specific for the use of the native organism. Rigid, durable, and solvent resistant, α-chitin is highly crystalline due to strong hydrogen bonding within chitin that forms an anti-parallel structure. The α-chitin is found in crustacean shells and fungal cell walls. Conversely the β-chitin, is less crystalline, contains weak hydrogen bonds, and forms a parallel configuration. As a result β-chitin is soft, pliable, and a water friendly material found in squid pens and pagonophore tubes [36].
2.2.1 Isolation of chitin

Isolation of chitin from crawfish shell wastes involves four traditional steps: demineralization, deproteinization, decolourization, and deacetylation. However, the isolation of chitin specifically consists of only two steps: demineralization and deproteinization. Several techniques to extract chitin from different sources have been reported. The most common method is referred to as the chemical procedure. The chemical method for isolation of chitin from crustacean shell biomass involves various major steps: elimination of inorganic matter (calcium carbonate) in dilute acidic medium (demineralization), and usually demineralization is accomplished by using HCl. Followed by extraction of protein matter in alkaline medium (deproteinization), and it is traditionally done by treating shell waste with aqueous solutions of NaOH or KOH.

The effectiveness of alkali deproteinization depends on the process temperature, the alkali concentration, and the ratio of its solution to the shells. As an alternative to the chemical process, a biological process using microorganisms has been evaluated for the demineralization and the deproteinization [36]. Recovery of the protein fraction of the shrimp waste by enzymatic hydrolysis has widely been investigated.
2.2.2 Properties of chitin

Chitin is a modified polysaccharide which contains nitrogen; it is synthesized from units of N-acetyl glucosamine (more precisely, 2-(acetyl amino)-2-deoxy-D-glucose). These units form covalent $\beta$-1,4-linkages (similar to the linkages between glucose units forming cellulose). Chitin may therefore be described as cellulose with one hydroxyl group on each monomer substituted with an acetyl amine group. This allows for increased hydrogen bonding between adjacent polymers, giving the chitin-polymer matrix increased strength. In its unmodified form, chitin is translucent, pliable, resilient and quite tough. In arthropods, however, it is often modified, becoming embedded in a hardened proteinaceous matrix, which forms much of the exoskeleton. In its pure form it is leathery, but when encrusted in calcium carbonate it becomes much harder.

2.3 Chitosan

2.3.1. History and discovery of chitosan

The history of chitosan (pronounced ky-toe-san) dates back to its first description by Branconnot in 1811. Rouget later discovered the deacetylated form of chitin, which was called chitosan, in 1859. The discovery of chitin is essentially based on some reactions carried out on raw material isolated from Agarics volvaceus, A. acris, A. cantarellus, A. piperatus, Hydnum repandum, H. hybridum and Boletus viscidus. According to Dodane and Vilivalam [37], Rouget further found that when chitin was boiled in a concentrated potassium hydroxide
solution, a product was obtained that dissolved in dilute iodine and acids, unlike chitin that only stained brown.

Chitin, chitosan and its derivatives have received much attention from scientists in different parts of the world. Due to its natural abundance and versatility, many investigations have focused on its properties and various applications. Although studies on chitin and chitosan were initiated in the early nineteenth century, most of the reports available today on its medical and pharmaceutical applications have been obtained only during the last couple of decades. Despite the considerable research carried out on chitosan over recent decades, new registered products have failed to reach the market.

2.3.2 Structure of chitosan

Chitosan (poly \([\beta-(1, 4)-2\text{-amino-2-deoxy-D-glucopyranose}]\)) is prepared by alkaline partial N-deacetylation of chitin. The molecular formula is \( \text{C}_6\text{H}_{11}\text{O}_{4}\text{N} \). The polymer is composed of copolymers of D-glucosamine (GlcN) and N-acetyl-D-glucosamine (GlcNAc). With regards to their chemical structure, chitin and chitosan have similar chemical structure. Chitin is made up of a linear chain of acetyl glucosamine groups while chitosan is obtained by removing enough acetyl groups (\(\text{CH}_3\text{-CO}\)) for the molecule to be soluble in most diluted acids. This process is called deacetylation. The actual difference between chitin and chitosan is the acetyl content of the polymer. Chitosan has one primary amino and two free hydroxyl groups for each C6 building unit, to form extensive intra and inter-molecular hydrogen bonding. Due to the easy availability of free amino
groups in chitosan, it carries a positive charge and thus in turn reacts with many negatively charged surfaces/polymers.

Chitosan is a one of the natural product of seafood obtained by chitin deacetylation, which is the second most abundant natural polymer. Its structure is very similar to that of cellulose except one of the hydroxyl groups are replaced by an amino group is shown in Figure 2.2 [38]. Chitosan can destroy bacteria by converting its amino group into an ammonium salt in dilute acid solutions. QAS can destroy the cell wall of the microorganism by connecting to its negatively charged protoplasm [39]. Chung et al. [40] used chitosan as an antibacterial finish along with a durable press finishing agent on 100% cotton fabrics and found that antibacterial activity remained to a level of 80% after 10 repeated launders.

Lee et al. [41] evaluated chitosan as an antibacterial agent along with a blood repellent finish. The researchers found that the treated cotton fabric showed higher reduction (97%) in the number of colonies of S. aureus bacteria compared to the number of colonies on a 55/45% wood pulp /polyester spun-laced nonwoven fabric. One of the problems with chitosan is wash fastness [42]. The finish does not last through repeated washing and is not regenerated.
Chitosan has received extensive consideration as an antimicrobial in textile materials. Studies indicated that cotton fabric coated with chitosan could produce up to a 99-100% reduction in S. aureus [43] remaining effective after fifty home laundering cycles [44]. Knill et al. [45] reported a method of effectively coating chitosan onto fibres for inclusion in wound dressings and led to good bacterial reductions. A similar approach but using a different technique was reported by Miraftab et al. [46] who spun alginate dope directly into chitosan solution as a coagulant for the sodium alginate.

2.3.3 Physico-chemical properties

Chitosan is actually a denomination describing a series of polymers with different degree of deacetylation and molecular weight. These two factors are very important for the physico-chemical properties of chitosan. It has been proposed to define chitosan and chitin as soluble or insoluble in 0.1 M acetic acid, respectively. The term chitosan is used to describe a series of polymers of
different degrees of deacetylation (DD), defined in terms of the percentage of
primary amino groups in the polymer backbone, and average molecular weights
(MW). Chitosan merely refers to a family of copolymers with various fractions of
acetylated units. It consists of two types of monomers, chitin-monomers and
chitosan-monomers. Commercial chitin and chitosan consists of both types of
monomers. The term chitosan embraces a series of polymers that vary in
average molecular weight and degree of deacetylation. Changing the reaction
conditions during the manufacture of chitosan from chitin can alter the DD and
MW of chitosan.

2.3.4 Degree of deacetylation

The process of deacetylation involves the removal of acetyl groups from
the molecular chain of chitin, leaving behind a compound (chitosan) with a high
degree chemical reactive amino group (-NH₂). This makes the degree of
deaacetylation (DD) an important property in chitosan production as it affects the
physicochemical properties, hence determines its appropriate applications [48].
Deacetylation also affects the biodegradability and immunological activity. A
sharp nomenclature border has not been defined between chitin and chitosan
based on the degree of N-deacetylation. In an earlier study by Rudall [47]
reviewed evidences suggesting that approximately one in every six to seven
residues in the chain has a proportion of free amino groups that manifests some
histochemical properties.
In any case, the degree of deacetylation can be employed to differentiate between chitin and chitosan because it determines the content of free amino groups in the polysaccharides. In fact there are two advantages of chitosan over chitin. In order to dissolve chitin, highly toxic solvents such as lithium chloride and dimethyl acetamide are used whereas chitosan is readily dissolved in diluted acetic acid. The second advantage is that chitosan possesses free amine groups, which are an active site in many chemical reactions. The degree of deacetylation of chitosan ranges from 50% to 99% with an average of 80%, depending on the crustacean species and the preparation methods. Chitin with a degree of deacetylation of 75% or above is generally known as chitosan.

2.3.5 Molecular weight

Chitosan is a biopolymer of high molecular weight. Like its composition, the molecular weight of chitosan varies with the raw material sources and the method of preparation. Molecular weight of native chitin is usually larger than one million Daltons while commercial chitosan products have the molecular weight range of 10,000 – 1,000,000 Daltons, depending on the process and grades of the product. Chitosan with MW of 250 - 500 kDa showed a maximum degradation temperature of approximately 280 °C, with low MW chitosan degrading at lower temperature, at 220 °C and 180 °C for MW 25 - 100 kDa and MW 2.5 - 5 kDa respectively. For instance at a temperature over 280°C, thermal degradation of chitosan occurs and polymer chains rapidly break down, thereby lowering molecular weight. Also, maximal depolymerisation caused by utilization
of high temperature or concentrated acids, such as hydrochloric acid followed by acetic acid and sulphurous acid, results in molecular weight changes with minimal degradation with the use of EDTA. The molecular weight of chitosan can be determined by methods such as chromatography, light scattering, and viscometry.

2.3.6 Solubility

Chitosan is a weak base, which is also hydrophilic. The D-glucosamine unit has a pKa value of 7.5. The basic nature of chitosan depends on its degree of deacetylation and apparent pKa value for the polymer is 6.5. It is estimated that deacetylation must be at least 85% complete in order to achieve the desired solubility. Low-molecular weight chitosan (MW<10 KDa) and chitosan salts may be more readily soluble, but high MW chitosan were occurred gel formation.

Chitosan is soluble in dilute and concentrated solutions of most organic acids and to some extent in mineral inorganic acids (including hydrochloric, lactic, glutamic and aspartic acids) that form water-soluble salts. Chitosan salts are soluble in water; the solubility depends on the degree of deacetylation and the pH of the solution. Upon dissolution, amine groups of the polymer become protonated rendering the molecule positively charged (R–NH$_3^+$). The concentration ratio between chitosan and acid is of great importance to impart desired functionality. The properties of chitosan can be modified by changing the degree of deacetylation and formulation properties such as the pH of solution
and ionic strength. If the pH of solution increased to close to neutral, most chitosan molecules will lose their charge and precipitation or gelation will occur.

2.3.7 Biological properties

In this respect, chitosan have attained increasing commercial interest as suitable resource materials due to their favourable biological properties including biocompatibility, non-toxicity and biodegradability. Several bioactive properties such as blood cholesterol control, weight loss effects, anti-inflammatory, anti-tumour activity, dental plaque inhibition, bone healing treatment, wound healing accelerator, hemostatic, anti-viral, antibacterial activity, antifungal activity, delivery of immunomodulatory agents have been reported.

Chitosan displayed a low oral toxicity with an LD50 in mouse of 16 g/kg bodyweight. Side effects of chitosan following oral administration relate to its effects within the gastrointestinal tract. Consumption of several grams of chitosan daily by humans can result in constipation or diarrhoea, because chitosan entraps water and lipids in the intestine. In man, in trials lasting for up to 12 weeks, no clinically significant adverse effects or changes in laboratory values relating to safety have been noted. Chitosan treatment had no effects on serum electrolyte levels or fat-soluble vitamin levels. The amounts of chitosan needed in pharmaceutical formulations are fairly low (less than 1000 mg/dose), and risks of side effects in the gastrointestinal tract are therefore also low.
Although chitosan is clinically well tolerated, it has been suggested that it might not be desirable for administration to individuals allergic to crustaceans, in which consumption of crustaceans frequently results in allergic reactions, and serious adverse reactions are possible. On the other hand, chitosan has been widely used in nonmedical natural products. No information about allergic reactions relating to these products has been available. Enzymatic hydrolysis of chitosan with lysozyme results in oligomer production whilst chitosanase gives dimer to pentamer products. $\beta$ - (1, 4) glycoside bond of chitosan will only be degraded by micro floral $\beta$-glycosidase in the lower part of the colon and result in non-toxic degradation products.

Properties such as biocompatibility, non-toxicity and biodegradability make chitosan an interesting material for biomedical and pharmaceutical applications. Also hypocholesterolemic, antimicrobial, antitumor and antiviral activity has been associated with chitosan [48]. The following discussion elaborates some of the before-mentioned properties in more detail.

### 2.3.8 Biocompatibility and toxicity

Chitosan is generally considered biocompatible with *in-vitro* and *in vivo*. This property is somewhat attributable to factors such as the natural source, molecular weight, DD and especially the preparation method [48].
2.3.9 Antimicrobial activity

Chitosan possesses antimicrobial activity towards a number of microorganisms such as bacteria, yeast, and fungi [48]. The antimicrobial activity of chitosan may be due to the interaction to the positively charged chitosan with anionic groups of bacteria cell surface. It has been suggested that this interaction creates an impermeable layer around the cell, which inhibits the transportation of essential solutes to the cell. Another antimicrobial mechanism is related to the inhibition of RNA and protein synthesis by permeation of chitosan into the cell nucleus. This mechanism is related to low molecular weight chitosan. Chitosan may also act as a chelating agent rendering trace elements, metals, and essential nutrients from the microorganism [49, 50]. Factors affecting the antibacterial activity of chitosan are DD, molecular weight, pH of the medium, and concentration in solution [51].

2.4 Preparation of chitosan

Although chitosan can be extracted from certain fungi, it is currently obtained mainly by the N-deacetylation of chitin, as this method is presently more convenient and less costly [52]. Chitosan can also be reacetylated from highly deacetylated chitosan’s [53]. The N-deacetylation of chitin is based on using highly concentrated aqueous or alcoholic alkali, normally sodium or potassium hydroxide (40-50%), at temperatures of 100-150 °C for periods of 1-5 h under heterogeneous conditions [54-55]. This treatment results in approximately 70%
deacetylated chitosan [56]. Repeating the procedure increases the degree of
deacetylation to a certain extent.

However, the molecular weight of the chitosan decreases with repeated
alkali treatment leading to a decrease in chain length. To overcome this problem,
the use of sodium thiophenolate has been suggested. Sodium thiophenolate
protects the polymer chains from degradation while simultaneous allowing the
catalysis of the reaction. To minimize the amount of NaOH used, also the mixing
of chitin with NaOH powder (weight ratio 1.5) by extrusion at 180ºC has been
proposed. This method results in highly deacetylated and soluble chitosan with
only one half of the NaOH needed for the aqueous treatment [57].

Figure 2.3 demonstrates the preparation process of chitosan, whereby the
acetamido group of chitin is deacetylated to an amino group due to alkali
treatment in the last stage of the process.

Chitin and chitosan can also be synthesized by enzymatic hydrolysis.
Examples of applicable enzymes include chitinases, chitosanases, lyzosomes
and / or cellulases [54-55]. Enzymatic synthesis usually leads to low molecular
weight chitosan (LMWC), or chitosan oligosaccharides (COS), that are readily
soluble in water due to shorter chain lengths and free amino groups in the D-
glucosamine units. This is considered as an advantage to insoluble chitosan, as
it is believed that solubility and low viscosity of the COS will increase the use of
chitosan in food and biomedical applications [59].
Figure 2.3: Preparation process of chitosan polymer
Enzymatic hydrolysis of chitin or chitosan is generally preferable to chemical hydrolysis associated with toxic compounds, environmental pollution and low production yields. However, the high cost of enzymatic hydrolysis restricts its use in COS production, although reuse of hydrolytic enzymes can decrease the production costs. Chitosan oligosaccharide synthesis can also be carried out using, for example, oxidative degradation and ultrasonic degradation [60]. The resulting physical forms after chitosan synthesis and processing usually include hydrogels, solutions, and solid state forms. Solid forms of chitosan include, for example, powders, flakes, particles, films, and fibres.

2.5 ZnO based materials

ZnO is a well-known semiconductor with a wide direct band gap (3.37 eV) and a large exciton binding energy of 60 meV at room temperature [61,62] and it has a wide range of applications such as solar cells, luminescent, electrical and acoustic devices, gas sensor and chemical sensors, coatings, catalysts, micro lasers, memory arrays and biomedical applications [63, 64].

Till now, many methods are used developed to synthesize zinc oxide nanocrystals including vapour phase growth [65], vapour-liquid- solid process [66], soft chemical method [67], electrophoretic deposition [68], sol-gel process [69], alcoholic hydrolysis of zinc precursors [70, 71], hydrothermal methods [72] and electrochemical routes [73].
2.5.1 Hydrothermal synthesis

Hydrothermal methods have been considered as excellent procedures for the preparation of crystalline nanoparticle metal oxides as the resulting particles have narrow size distribution, good crystallization, phase purity, and comparatively few agglomerates. Hydrothermal methods also have low growth temperature and simple process control [74].

Another similar wet chemical process to prepare metal oxides thin films or coatings is solvothermal method, in which organic solvent, instead of water in hydrothermal method, is used [75–78]. Zhang et al. used ethylene glycol as the solvent to synthesize 3D hallow micro hemisphere ZnO [79]. Yang et al. used ethanol as the solvent to synthesize single crystal ZnO nanorods at a low temperature of 80°C [78]. Hydrothermal growth of ZnO nanowires using water solvent was first demonstrated by Vayssieres [79, 80]. Vayssieres indicated that lowering the overall concentration of reagents was the easiest way to obtain anisotropic ZnO nanoparticles.

Among these techniques, the hydrolysis route is very attractive because it is relatively easy to perform and allows us to tailor the morphology of the particles by controlling the rate of hydrolysis and condensation reactions [82]. Spanhel and Anderson [81] have explained the synthesis of nanocrystals of ZnO using distillation set-up starting with product of zinc acetate and ethanol. They
have obtained highly concentrated colloidal nanocrystals of ZnO of size varying from 3.5 to 5.5 nm. Hossain et al. [83] have further modified this technique to obtain nanobelts of ZnO of length 700 mm using refluxing technique.

Several workers have used capping agent such as vinyl pyridine (PVP), poly ethylene glycol (PEG), etc., to stop particle agglomeration and obtained nanoparticles of size less than 5 nm [84, 85]. Much research has been focused on the preparation and the properties of ZnO nanocrystals; however, little of it dealt with the pH effect of the sol on the crystallite size of ZnO powder. For instance, Li et al. [86] concluded that the solution conditions have a certain effect on the particle size of ZnO powders under hydrothermal conditions. Zhang et al. [87] found that the pH value can change the quantity of ZnO nuclei and of growth units. Lu and Yeh [88] found that the characteristics of ZnO powder profoundly depend on the pH of the starting solutions. In addition the crystallinity and particle size of ZnO powder increase with arise in the pH of solution.

2.5.2 Sol-gel synthesis

The growth of ZnO from zinc acetate dihydrate precursor using sol–gel process generally undergoes four stages, such as solvation, hydrolysis, polymerization and transformation into ZnO. The zinc acetate dihydrate precursor was first solvated in ethanol, and then hydrolyzed, regarded as removal of the intercalated acetate ions and results in a colloidal–gel of zinc hydroxide (Eq. (5)), size and activity of solvent have obvious influence on the reacting progress and product. Ethanol has smaller size and a more active –OH. Ethanol can react more easily to form a polymer precursor with a higher polymerization degree,
which is required to convert sol into gel [89]. These zinc hydroxide splits into Zn$^{2+}$
cation and OH$^-$ anion according to reactions (Eq. (4)) and followed by
polymerization of hydroxyl complex to form “Zn–O–Zn” bridges and finally
transformed into ZnO as showed in the Eq. (5) [90] :

$$[\text{CH}_3\text{-CO (H}_2\text{O)}\text{-O-Zn-O-CO (H}_2\text{O)-CH}_3] + [\text{N (CH}_2\text{-CH}_2\text{-OH)}_3] \rightarrow$$

$$[\text{CH}_3\text{-CO (H}_2\text{O)}\text{-O-Zn-O-CO (H}_2\text{O)-CH}_3] \rightarrow$$

$$(\text{CH}_3\text{-CO-O-Zn})^+ + [\text{O-CO-CH}_3]^+ + 2\text{H}^+ + 2\text{OH}^-$$  \hspace{1cm} (2)

$$(\text{CH}_3\text{-CO-O-Zn})^+ + [\text{N (CH}_2\text{-CH}_2\text{-OH)}_3] \rightarrow$$

$$(\text{CH}_3\text{-CO-O-Zn) N (CH}_2\text{-CH}_2\text{-OH)}_2 + (\text{CH}_2\text{-CH}_2\text{-OH})$$  \hspace{1cm} (3)

$$\text{Zn (OAc)}_2 \rightarrow 2\text{Zn}^{2+} + 2(\text{OAc})^-$$  \hspace{1cm} (4)

$$\text{Zn}^{2+} + 2\text{OH}^- \rightarrow \text{Zn (OH)}_2 \rightarrow \text{ZnO} + \text{H}_2\text{O}$$  \hspace{1cm} (5)

2.5.3 Effect of pH on ZnO

A lot of factors come into play during the growth of the ZnO nanorods like
concentration of the chemical bath, temperature, duration of growth, pH, etc.,
which directly affect the final morphology of the rods grown. There are reports
available in the literature about the synthesis of ZnO nanorods and other
morphologies through a variation in pH of the reaction bath [91-93].

However, this study is aimed at optimizing the pH conditions to obtain ZnO
nanorods of different dimensions starting with the same concentration of the
reactant mixture. It was possible to grow ZnO nanorods of different dimensions (both lateral and longitudinal), with the same concentration of Zn (NO₃)₂ and hexamine in the chemical bath and the same growth duration, simply by varying the pH of the growth solution between 6 and 7.3.

### 2.5.4 Wet chemical process

A small amount of industrial production involves wet chemical processes, which start with aqueous solutions of purified zinc salts, from which zinc carbonate or zinc hydroxide is precipitated. The precipitate is then filtered, washed, dried and calcined at temperatures around 800°C.

### 2.5.5 Laboratory synthesis

Synthetic ZnO crystals, red and green colours are associated with different concentrations of oxygen vacancies [94]. A large number of specialized methods exist for producing ZnO for scientific studies and niche applications. These methods can be classified by the resulting ZnO form (bulk, thin film, nanowire), temperature ("low", that is close to room temperature or "high", that is T ~ 1000°C), process type (vapour deposition or growth from solution) and other parameters.

Large single crystals (many cubic centimetres) are usually grown by the gas transport (vapour-phase deposition), hydrothermal synthesis, [94, 95 & 96] or melt growth [97]. However, because of high vapour pressure of ZnO, growth from
the melt is problematic. Growth by gas transport is difficult to control, leaving the hydrothermal method as a preference [97]. Thin films can be produced by chemical vapour deposition, metal organic vapour phase epitaxial, electrodeposition, pulsed laser deposition, sputtering, sol-gel synthesis, atomic layer deposition, spray pyrolysis, etc.

Ordinary white powdered zinc oxide can be produced in the laboratory by electrolyzing a solution of sodium bicarbonate with a zinc anode. Zinc hydroxide and hydrogen gas are produced. The zinc hydroxide upon heating decomposes to zinc oxide.

\[
\begin{align*}
\text{Zn} & + 2 \text{H}_2\text{O} \rightarrow \text{Zn(OH)}_2 + \text{H}_2 \\
\text{Zn(OH)}_2 & \rightarrow \text{ZnO} + \text{H}_2\text{O}
\end{align*}
\]

### 2.5.6 Chemical properties

ZnO occurs as a white powder. The mineral zincite usually contains manganese and other impurities that confer a yellow to red colour [98]. Crystalline zinc oxide is thermochromic, changing from white to yellow when heated and in air reverting to white on cooling [99]. This colour change is caused by a small loss of oxygen to the environment at high temperatures to form the non-stoichiometric \( \text{Zn}_{1+x}\text{O} \), where at 800 °C, \( x = 0.00007 \) [99].

Zinc oxide is an amphoteric oxide. It is nearly insoluble in water, but it is soluble in (degraded by) most acids, such as hydrochloric acid [100, 101].
\[ \text{ZnO} + 2 \text{HCl} \rightarrow \text{ZnCl}_2 + \text{H}_2\text{O} \]

Bases also degrade the solid to give soluble zincates:

\[ \text{ZnO} + 2 \text{NaOH} + \text{H}_2\text{O} \rightarrow \text{Na}_2[\text{Zn(OH)}_4] \]

\( \text{ZnO} \) reacts slowly with fatty acids in oils to produce the corresponding carboxylates, such as oleate or stearate. \( \text{ZnO} \) forms cement-like products when mixed with a strong aqueous solution of zinc chloride and these are best described as zinc hydroxyl chlorides [102]. This cement was used in dentistry [103].

\( \text{ZnO} \) also forms cement-like products when treated with phosphoric acid; related materials are used in dentistry [101]. A major component of zinc phosphate cement produced by this reaction is hopeite, \( \text{Zn}_3(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O} \) [105].

\( \text{ZnO} \) decomposes into zinc vapour and oxygen only at around 1975°C, reflecting its considerable stability. Heating with carbon converts the oxide into the metal, which is more volatile than the oxide [106].

\( \text{ZnO} \) can react violently with aluminium and magnesium powders, with chlorinated rubber and linseed oil on heating causing fire and explosion hazard [107, 108]. It reacts with hydrogen sulphide to give the sulphide. This reaction is used commercially in removing \( \text{H}_2\text{S} \) using \( \text{ZnO} \) powder (e.g., as deodorant) [101].
### 2.5.7 Physical Properties

<table>
<thead>
<tr>
<th>Properties</th>
<th>Characteristic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occurrence</td>
<td>Zinc oxide (zincite) rarely occurs in nature, particularly in a crystalline form. It is usually orange / red in colour due to manganese impurity</td>
</tr>
<tr>
<td>Crystal Structure</td>
<td>wurtzite hexagonal crystal structure</td>
</tr>
<tr>
<td>Molecular Weight</td>
<td>81.37</td>
</tr>
<tr>
<td>Colour</td>
<td>Pure microcrystalline zinc oxide is white</td>
</tr>
<tr>
<td>Relative Density</td>
<td>5.607</td>
</tr>
<tr>
<td>Melting Point</td>
<td>Zinc oxide sublimes at atmospheric pressure at temperatures over 1200°C. Under high pressure a melting point of 1975°C has been estimated</td>
</tr>
<tr>
<td>Vapour Pressure</td>
<td>$\cong 1500^\circ C = 12 \text{ mm.}$</td>
</tr>
<tr>
<td>Refractive Index</td>
<td>$\omega = 2.004$, $\epsilon = 2.020$</td>
</tr>
<tr>
<td>Heat Capacity</td>
<td>$C_p = 9.62 \text{ cal / deg / mole} \cong 25^\circ C$</td>
</tr>
<tr>
<td>Heat</td>
<td>$\Delta H = -83.25 \text{ Kcal / deg / mole} \cong 25^\circ C$</td>
</tr>
<tr>
<td>Free energy of formation</td>
<td>$\Delta F = -76.1 \text{ Kcal / deg C / mole} \cong 25^\circ C$</td>
</tr>
<tr>
<td>Coefficient of Thermal Expansion</td>
<td>$4 \times 10^{-6} / \text{ deg C}$</td>
</tr>
<tr>
<td>Electrical Conductivity</td>
<td>In its normal form, zinc oxide is an n-type semiconductor</td>
</tr>
<tr>
<td>Rectification</td>
<td>Single crystals of zinc oxide can act as rectifiers. This is the origin of ‘crystal sets’, very early radio receivers.</td>
</tr>
<tr>
<td>Optical Properties</td>
<td>Zinc oxide is transparent to visible light but strongly absorbs ultra violet light below 3655 A.</td>
</tr>
</tbody>
</table>
2.5.7.1 Mechanical properties

ZnO is a relatively soft material with approximate hardness of 4.5 on the Mohs scale [114]. Its elastic constants are smaller than those of relevant III-V semiconductors, such as GaN. The high heat capacity and heat conductivity, low thermal expansion and high melting temperature of ZnO are beneficial for ceramics [115]. ZnO is most stable phase being wurtzite, ZnO exhibits a very long lived optical phonon $E_2$ (low) with a life time as high as 133 ps at 10K [116]. Among the tetrahedrally bonded semiconductors, it has been stated that ZnO has the highest piezoelectric tensor or at least one comparable to that of GaN and AlN [117]. This property makes it a technologically important material for many piezoelectrical applications, which require a large electromechanical coupling.

2.5.7.2 Crystal structure

![Wurtzite structure](image1.png) ![Zinc blende unit cell](image2.png)

*Figure 2.4: Basic structures of ZnO (a) Crystal structure and (b) Unit cell*
Zinc oxide crystallizes in two main forms, hexagonal wurtzite [109] and cubic zinc blende unit cell as shown in Figure 2.4. The wurtzite structure is most stable at ambient conditions and thus most common. The zinc blende form can be stabilized by growing ZnO on substrates with cubic lattice structure. In both cases, the zinc and oxide centers are tetrahedral, the most characteristic geometry for Zn (II).

In addition to the wurtzite and zinc blende polymorphs, ZnO can be crystallized in the rock salt motif at relatively high pressures about 10 GPA [110]. Hexagonal and zinc blende polymorphs have no inversion symmetry (reflection of a crystal relative to any given point does not transform it into itself). This and other lattice symmetry properties result in piezoelectricity of the hexagonal and zinc blende ZnO, and pyroelectricity of hexagonal ZnO.

The hexagonal structure has a point group 6 mm (Hermann-Mauguin notation) or C$_{6v}$ (Schoenflies notation), and the space group is P6$_3$mc or C$_{6v}^4$. The lattice constants are $a = 3.25$ Å and $c = 5.2$ Å; their ratio $c/a \sim 1.60$ is close to the ideal value for hexagonal cell $c/a = 1.633$ [111] As in most group II-VI materials, the bonding in ZnO is largely ionic (Zn$^{2+}$–O$^{2-}$) with the corresponding radii of 0.074 nm for Zn$^{2+}$ and 0.140 nm for O$^{2-}$. This property accounts for the preferential formation of wurtzite rather than zinc blende structure, [112] as well as the strong piezoelectricity of ZnO. Because of the polar Zn-O bonds, zinc and oxygen planes are electrically charged.
To maintain electrical neutrality, those planes reconstruct at atomic level in most relative materials, but not in ZnO – its surfaces are atomically flat, stable and exhibit no reconstruction. This anomaly of ZnO is not fully explained yet [113].

### 2.5.8 Electrical properties

ZnO has a relatively large direct band gap of ~3.3 eV at room temperature. Advantages associated with a large band gap include higher breakdown voltages, ability to sustain large electric fields, lower electronic noise, and high-temperature and high-power operation. The band gap of ZnO can further be tuned to ~3 - 4 eV by its alloying with magnesium oxide or cadmium oxide [110].

#### 2.5.8.1 n-type doping

Most ZnO has n-type character, even in the absence of intentional doping. Non-stoichiometry is typically the origin of n-type character, but the subject remains controversial [118]. An alternative explanation has been proposed, based on theoretical calculations, that unintentional substitution hydrogen impurities are responsible [119]. Controllable n-type doping is easily achieved by substituting Zn with group-III elements such as Aluminium, Gallium and Indium or by substituting oxygen with group-VII elements chlorine or iodine [120].
2.5.8.2 p - Type doping

Reliable p-type doping of ZnO remains difficult. This problem originates from low solubility of p-type dopants and their compensation by abundant n-type impurities. This problem is observed with GaN and ZnSe. Measurement of p-type in "intrinsically" n-type material is complicated by the inhomogeneity of samples [121].

Current limitations to p-doping do not limit electronic and optoelectronic applications of ZnO, which usually require junctions of n-type and p-type material. Known p-type dopants include group-I elements Li, Na, K; group-V elements N, P and As; as well as copper and silver. However, many of these form deep acceptors and do not produce significant p-type conduction at room temperature [111].

Electron mobility of ZnO strongly varies with temperature and has a maximum of ~2000 cm²/(V·s) at 80K [122]. Data on hole mobility are scarce with values in the range 5 – 30 cm²/(V·s) [123].

2.6 Chitosan- metal complexes

Recently, Wang et al. [124] were prepared five chitosan–zinc complexes with different zinc content and characterized FT-IR, XRD, AAS and elemental analysis. It was found that the complexes with different zinc content had different molecule structure. Three possible structures corresponding to different chelate
ratios were proposed and preliminarily complexation mechanisms were discussed (Figure 2.5).

In vitro antimicrobial activities of complexes were evaluated against 11 species of bacteria and fungi. The complexes showed wide spectrum of effective antimicrobial activities, which were 2-8 times and 4-16 times higher than those of chitosan and zinc sulphate, respectively, and improved with increasing content of zinc ions. The complexes had a better antibacterial activity than antifungal activity, and showed excellent activity particularly against *E. coli* and *Corynebacterial* both with a MIC value of 0.000313% (CS–Zn w/v).

![Diagram of chitosan-Zn complexes](image)

**Figure 2.5: Proposed mechanism of chitosan-Zn complexes**
2.6.1 Chitosan-TiO$_2$ composite

Novel chitosan/nano-TiO$_2$ composite emulsion was prepared with chitosan and nano-TiO$_2$ by inverse suspension technology by Shi et al. [125]. The antibacterial abilities of gauze treated with chitosan/nano-TiO$_2$ composite emulsion were studied. The results showed that the antibacterial gauze had excellent antibacterial ability under invisible light. The gauze treated with chitosan/nano-TiO$_2$ composite emulsion showed broad-spectrum and stabilized antibacterial properties against *E. coli*, *A. Niger* and *C. albicans*. And the antibacterial activity was hardly influenced with the increase of contamination time or storage time. Using the excellent functions of the gauze to explore green textiles would be a new development.

2.6.2 Chitosan / ZnO nanoparticles composite

Novel chitosan/ZnO nanoparticles composite membranes were successfully prepared via a new method of sol-cast transformation by Li et al. [11]. The product exhibited good mechanical properties and high antibacterial activities. And CS/nano-ZnO composite membranes with 6-10 wt. % ZnO showed high antibacterial activities. At the same time, ZnO content had effect on the mechanical properties of the composite membranes. When doped with 6 wt. % ZnO, CS/nano-ZnO composite membranes showed good antibacterial and mechanical properties. It suggested that such CS/nano-ZnO composite membranes had potential application as medical materials.
2.6.3 Chitosan/n-ZnO for dye absorption

The preparation, characterization and dye adsorption properties of novel biocompatible composite (Chitosan–zinc oxide nanoparticle) (CS/n-ZnO) were investigated by Salehi et al. [12]. CS/n-ZnO was studied using Fourier transform infra-red (FT-IR), X-ray diffraction (XRD), scanning electron microscopy (SEM) and wavelength dispersive X-ray spectroscopy (WDX). Equilibrium and kinetic studies were done for the adsorption of Direct Blue 78 (DB78) and Acid Black 26 (AB26) from aqueous solutions onto CS/n-ZnO. Results showed that zinc oxide nanoparticles were immobilized onto Chitosan. Adsorption studies showed that CS/n-ZnO could be effectively used as a biocompatible composite adsorbent for the removal of anionic dyes. The adsorption kinetics studies of dyes on CS/n-ZnO were performed based on pseudo-first order, pseudo-second order and intraparticle diffusion rate mechanism. The data indicated that the adsorption kinetics of dyes on CS/n-ZnO followed the pseudo-second order.

The equilibrium data have been analyzed using Langmuir, Freundlich and Tempkin isotherms. It was found that data for AB26 and DB78 followed with Langmuir and Tempkin isotherms, respectively. The results showed that the CS/n-ZnO being a biocompatible, eco-friendly and low-cost adsorbent with relatively large adsorption capacity might be a suitable alternative for elimination of dyes from colour aqueous solutions.
2.7 Graphite

The graphite is an allotrope of carbon. It was named by Abraham Gottlob Werner in 1789 from the Ancient Greek. Graphite is use in pencils, where it is commonly called lead (not to be confused with the metallic element lead). Unlike diamond (another carbon allotrope), graphite is an electrical conductor, a semimetal. It is, consequently, useful in such applications as arc lamp electrodes.

Graphite is the most stable form of carbon under standard conditions. Therefore, it is used in thermochemistry as the standard state for defining the heat of formation of carbon compounds. Graphite may be considered the highest grade of coal, just above anthracite and alternatively called meta-anthracite, although it is not normally used as fuel because it is difficult to ignite.

2.7.1 Graphite structure

The crystalline structure of graphite (Figure 2.6) consists of hexagonal rings forming thin parallel plates (graphene). Each carbon atom is covalently bonded to three other atoms in the plate (the angle between two bonds is 120°).

The outermost electron shell of a carbon atom has four valence electrons, three of which are used by the covalent bonds. The forth valence electron does not take part in covalent bonds and may be easily displaced from the electron shell by an electric field. These electrons provide electrical conductivity of graphite.
The graphene are bonded to each other by weak Van der Waals forces. The layered structure of graphite allows sliding movement of the parallel graphene plates. Weak bonding between the plates determines softness and self-lubricating properties of graphite. Graphite is rarely found in form of monocrystals. Most of graphite occurs in form of flakes or lumps. Graphite material having fine Grain structure is sometimes named amorphous graphite; however it is not really amorphous but microcrystalline.
2.8 Graphene

Graphene is a substance made of pure carbon, with atoms arranged in a regular hexagonal pattern similar to graphite, but in a one-atom thick sheet. It is very light, with a 1 square meter sheet weighing only .77 milligrams. It is an allotrope of carbon whose structure is a single planar sheet of sp2-bonded carbon atoms that are densely packed in a honeycomb crystal lattice [126]. The term graphene was coined as a combination of graphite and the suffix (–ene) by Hans - Peter Boehm [127]. Who described single-layer carbon foils in 1962 [128]. Graphene is most easily visualized as an atomic-scale chicken wire made of carbon atoms and their bonds. The crystalline or "flake" form of graphite consists of many graphene sheets stacked together. The structure of graphene is shown in Figure 2.7.

The carbon-carbon bond length in graphene is about 0.142 nanometres [129]. Graphene sheets stack to form graphite with an interplanar spacing of 0.335 nm. Graphene is the basic structural element of some carbon allotropes including graphite, charcoal, carbon nanotubes and fullerenes. It can also be considered as an indefinitely large aromatic molecule, the limiting case of the family of flat polycyclic aromatic hydrocarbons.
2.8.1 Exfoliated graphene

In 2004, the Manchester group obtained graphene by micro-mechanical alleviation of graphite. They used adhesive tape to repeatedly split graphite crystals into increasingly thinner pieces (however, filed in 2002 US Patent 6,667,100 describes the process in detail to achieve a graphite thickness of 0.01 thousands of an inch). The tape with attached optically transparent flakes was
dissolved in acetone, and, after a few further steps, the flakes including monolayers were sediment on a silicon wafer. Individual atomic planes were then hunted in an optical microscope.

A year later, the researchers simplified the technique and started using dry deposition, avoiding the stage when graphene floated in a liquid. Relatively large crystallites (first, only a few micrometres in size but, eventually, larger than 1 mm and visible by the naked eye) were obtained by the technique. It is often referred to as a scotch tape or drawing method. The latter name appeared because the dry deposition resembles drawing with a piece of graphite [130]. The key for the success probably was the use of high-throughput visual recognition of graphene on a properly chosen substrate, which provides a small but noticeable optical contrast. The optical properties section below contains a photograph of what graphene looks like.

The isolation of graphene led to the current research boom. Previously, free-standing atomic planes were often "presumed not to exist" [131] because they are thermodynamically unstable on a nanometre scale [132] and, if unsupported, have a tendency to scroll and buckle [133]. It is currently believed that intrinsic microscopic roughening on the scale of 1 nm could be important for the stability of purely 2D crystals [134].

There were a number of previous attempts to make atomically thin graphitic films by using exfoliation techniques similar to the drawing method.
Multilayer samples down to 10 nm in thickness were obtained. These efforts were reviewed in 2007 [126]. Furthermore, a couple of very old papers were recently unearthed [135] in which researchers tried to isolate graphene starting with intercalated compounds. These papers reported the observation of very thin graphitic fragments (possibly monolayers) by transmission electron microscopy. Neither of the earlier observations was sufficient to "spark the graphene gold rush", until the Science paper did so by reporting not only macroscopic samples of extracted atomic planes but, importantly, their unusual properties such as the bipolar-transistor effect, ballistic transport of charges, large quantum oscillations, etc.

The discovery of such interesting qualities intrinsic to graphene gave an immediate boost to further research and several groups quickly repeated the initial result and moved further. These breakthroughs also helped to attract attention to other production techniques, such as epitaxial growth of ultra-thin graphitic films. In particular, it has later been found that graphene monolayers grown on silicon carbide and Iridium are weakly coupled to these substrates (how weakly remains debated) and the graphene–substrate interaction can be passivated further [136].

Not only graphene but also free-standing atomic planes of boron nitride, mica, dichalcogenides and complex oxides were obtained by using the drawing method [137]. Unlike graphene, the other 2D materials have so far attracted surprisingly little attention.
2.8.2 Electronic properties

Graphene differs from most conventional three-dimensional materials. Intrinsic graphene is a semi-metal or zero-gap semiconductor. Understanding the electronic structure of graphene is the starting point for finding the band structure of graphite. It was realized as early as 1947 by P. R. Wallace [138] that the E-k relation is linear for low energies near the six corners of the two-dimensional hexagonal Brillouin zone, leading to zero effective mass for electrons and holes [139].

Due to this linear (or "conical") dispersion relation at low energies, electrons and holes near these six points, two of which are in equivalent, behave like relativistic particles described by the Dirac equation for spin 1/2 particles [140,141]. Hence, the electrons and holes are called Dirac fermions also called Graphinos, [142] and the six corners of the Brillouin zone are called the Dirac points [140]. The equation describing the E-k relation is: where the Fermi velocity \( v_F \sim 10^6 \text{ m/s} \) [141].

2.8.3 Optical properties

Graphene is unique optical properties produce an unexpectedly high opacity for an atomic monolayer, with a startlingly simple value: it absorbs \( \pi \alpha \approx 2.3\% \) of white light, where \( \alpha \) is the fine-structure constant [143]. This is "a consequence of the unusual low-energy electronic structure of monolayer
graphene that features electron and hole conical bands meeting each other at the Dirac point, which is qualitatively different from more common quadratic massive bands" [144]. Based on the Slonczewski-Weiss-McClure (SWMcC) band model of graphite, the interatomic distance, hopping value and frequency cancel when the optical conductance is calculated using the Fresnel equations in the thin-film limit.

This has been confirmed experimentally, but the measurement is not precise enough to improve on other techniques for determining the fine-structure constant [145]. The band gap of graphene can be tuned from 0 to 0.25 eV (about 5 micrometre wavelength) by applying voltage to a dual-gate bilayer graphene field-effect transistor (FET) at room temperature [146].

The optical response of graphene nano ribbons has also been shown to be tunable into the terahertz regime by an applied magnetic field [147]. It has been shown that graphene/graphene oxide system exhibits electrochromic behaviour, allowing tuning of both linear and ultrafast optical properties [148].

Recently, a graphene-based Bragg grating (one-dimensional photonic crystal) has been fabricated and demonstrated its competence for excitation of surface electromagnetic waves in the periodic structure using prism coupling technique [149].
2.9 Graphene Oxide

Graphite oxide (GO), formerly called graphitic oxide or graphitic acid, is a compound of carbon, oxygen, and hydrogen in variable ratios, obtained by treating graphite with strong oxidizers. The maximally oxidized bulk product is a yellow solid with C: O ratio between 2.1 and 2.9, that retains the layer structure of graphite but with a much larger and irregular spacing [12].

The bulk material disperses in basic solutions to yield monomolecular sheets, known as graphene oxide by analogy to graphene, the single-layer form of graphite [148]. Graphene oxide (GO) sheets have been used to prepare a strong paper-like material, and have recently attracted substantial interest as a possible intermediate for the manufacture of graphene. However, as of 2010 this goal remains elusive since graphene obtained by this route still has many chemical and structural defects.

2.9.1 Structure

The structure and properties of graphite oxide depend on particular synthesis method and degree of oxidation. It typically preserves the layer structure of the parent graphite, but the layers are buckled and the interlayer spacing is about two times larger (~0.7 nm) than that of graphite. Strictly speaking "oxide" is an incorrect but historically established name. Besides oxygen epoxide groups (bridging oxygen atoms), other functional groups experimentally found are: carbonyl (=CO), hydroxyl (-OH), phenol groups...
attached to both sides [151, 152]. There is evidence of "buckling" (deviation from planarity), folding and cracking of graphene oxide sheets upon deposition of the layers on a choice of substrate. The detailed structure is still not understood due to the strong disorder and irregular packing of the layers (Figure 2.8).

![Figure 2.8: Atomic structure of Graphene Oxide](image)

Graphene oxide layers are about 1.1 ± 0.2 nm thick [152, 153]. Scanning tunnelling microscopy shows the presence of local regions where oxygen atoms are arranged in a rectangular pattern with lattice constant 0.27 nm × .41 nm [154, 155]. The edges of each layer are terminated with carboxyl and carbonyl groups [156].
X-ray photoelectron spectroscopy shows the presence of carbon atoms in non-oxygenated ring contexts (284.8 eV), in C-O (286.2 eV), in C=O (287.8 eV) and in O-C=O (289.0 eV) [158].

Graphite oxide is easily hydrated, resulting in a distinct increase of the inter-planar distance (up to 1.2 nm in saturated state). Additional water is also incorporated into interlayer space due to high pressure induced effects [158]. The bulk product absorbs moisture from ambient air proportionally to humidity. Complete removal of water from the structure seems difficult since heating at 60–80°C results in partial decomposition and degradation of the material. Similar to water, graphite oxide also easily incorporates other polar solvents, e.g. alcohol. Separation of graphite oxide layers is proportional to the size of alcohol molecule; additional monolayer is inserted into the structure at high pressure conditions [159].

Graphite oxide exfoliates and decomposes when rapidly heated at moderately high temperatures (~280–300°C) with formation of finely dispersed amorphous carbon, somewhat similar to activated carbon [160].

2.9.2 Chitosan/GO nanocomposites

Chitosan/GO nanocomposites [161] prepared via simple and environmentally friendly strategy for the synthesis of CS/GO nanocomposites by self-assembly. It is crucial to have the uniform filler dispersion within the polymer matrix and good interfacial adhesion between nanofillers and polymer matrix. In the family of graphene, GO attaches many oxygen-containing hydrophilic
functional groups such as -COOH and -OH [162-163]. Moreover, the surfaces of GO sheets are highly negatively charged when dispersed in water, apparently as a result of ionization of carboxylic acid and phenolic hydroxyl groups on the GO sheets [165]. The hydrophobicity of GO and electrostatic repulsion results in the dispersion of GO at the individual sheet level in water. On the other hand, as a hydrophilic biopolymer with -NH$_2$ and -OH in each unit, CS can be protonated to polycationic material in acid media, which is favour of the interaction between polymer chains and GO sheets. Thus a good dispersion of GO in CS solution is expected. Here we report a simple and environmentally friendly strategy for the synthesis of CS/GO nanocomposites by self-assembly. The tensile strength and Young's modulus are significantly improved by about 122 and 64%, respectively, by addition of only 1 wt % GO.

Well-dispersed CS/GO nanocomposites were fabricated through a simple self-assembly method by solution mixing as described in the experimental section. GO can be dispersed very well in water at the level of individual sheets because of the many oxygen-containing functional groups on the surfaces of GO and electrostatic repulsion between the negative charge of GO sheets. Furthermore, because of the many amino and hydroxyl groups in the unit of CS and the polycationic nature of CS in acid media, electrostatic attraction and hydrogen bonding between CS and GO are potentially achievable and could induce the truly homogeneous co-dispersion of CS and GO on the molecular scale and enhance interfacial adhesion as well as mechanical performance of the nanocomposite [166].
In general, good dispersion and interfacial stress transfer are important factors for repairing reinforcing nanocomposites. This leads to a more uniform stress distribution and minimizes the presence of the stress concentration centre [167]. As discussed above, the oxygen-containing groups and negative charges on the GO surface can interact effectively with the polycationic CS through hydrogen bonding and electrostatic attraction. Moreover, the large aspect ratio of the graphene sheets is also favourable to stress transfer. The compatibility and strong interaction between GO and the CS matrix greatly enhances the unidirectional dispersion of GO sheets on the molecular scale in CS matrix as well as the interfacial adhesion, thus significantly increasing the mechanical properties of the nanocomposites.

Biopolymer nanocomposites were prepared from CS as the matrix and GO as reinforcing nanofillers by a simple self-assembly strategy. A uniform distribution and fine dispersion for GO in CS matrix have been evidenced. The incorporation of only 1 wt% GO dramatically increases the tensile strength and Young’s modulus by 122 and 64%, respectively, from 40.1 to 89.2 MPa and 1.32 to 2.17 GPA [168-171]. Meanwhile, the elongation at the break point increases significantly. Our work demonstrates a good example for the preparation of high-performance polymer nanocomposites by using nanofiller graphene. And it can be expected that CS with largely improved mechanical properties may play a more important role in biochemical and electrochemical applications.
From the above overviews of recent literature, it is clear that there are only few studies on chitosan-metal, chitosan-metal oxide composites and chitosan-graphene oxide composites. Due to the importance of composites for many applications, attention has to be focused upon the development of suitable chitosan based hybrid composite.

2.10 Conclusion

A number of studies on the preparation of chitosan-ZnO composites using precipitation methods with different metal combinations such as chitosan-metal complexes [124], chitosan-TiO$_2$ composites [125], Chitosan-n-ZnO membrane [11], Chitosan-nano ZnO composite [12] and Chitosan treated with graphene oxide [161] and Graphene oxide was treated with ZnO [171], were reported in the literature. To our knowledge, there is no report on the preparation and characterization of chitosan-ZnO nanostructures and its graphene oxide hybrid composites.