Biopolymer chitin, the most abundant natural amino polysaccharide, and its most important derivative, chitosan, are recently considered as the subjects for extensive worldwide academic and industrial research. In spite of potential applications of chitin and chitosan, it is necessary to establish efficient appropriate modifications to explore fully their high potential. A variety of chemical modifications are employed to modify these carbohydrate polymers. The present article provides a comprehensive review on one of the most promising approaches to modify chitin and chitosan, i.e., graft copolymerization, with an emphasis on the synthetic aspects. Both chemically- and radiation-initiated graft copolymerization of various vinyl monomers onto the trunk polymers are investigated. Meanwhile, the limited cases of polycondensation and oxidative coupling are presented as the non-vinyl graft copolymerization methods. Then, the ring-opening graft copolymerization is described and the cases of the cyclic monomers \(\alpha\)-aminoacid \(N\)-carboxy anhydrides and \(\varepsilon\)-caprolactone are investigated. An extensive description of the “grafting onto” approach is provided. The preformed polymers discussed here for grafting onto chitin/chitosan include living poly(2-alkyl oxazolines), poly(ethylene glycol)s, block polyethers, poly(ethylene imine)s, poly(2-hydroxyalkanoate)s, polyurethanes, poly(dimethylsiloxane)s, and dendrimer-like hyperbranched polymers. Chitin/chitosan multiple modification including graft copolymerization is also investigated. Regioselective grafting using derivatives such as 6-iodo-, mercapto-, deoxy(thiosulphato)-chitins, and \(N\)-trichloroacetyl chitosan are described as suitable approaches to achieve chitin/chitosan graft copolymers with well-known structures.

KEYWORDS: chitin; chitosan; polysaccharide; graft copolymerization; modification.

ABSTRACT

B iopolymer chitin, the most abundant natural amino polysaccharide, and its most important derivative, chitosan, are recently considered as the subjects for extensive worldwide academic and industrial research. In spite of potential applications of chitin and chitosan, it is necessary to establish efficient appropriate modifications to explore fully their high potential. A variety of chemical modifications are employed to modify these carbohydrate polymers. The present article provides a comprehensive review on one of the most promising approaches to modify chitin and chitosan, i.e., graft copolymerization, with an emphasis on the synthetic aspects. Both chemically- and radiation-initiated graft copolymerization of various vinyl monomers onto the trunk polymers are investigated. Meanwhile, the limited cases of polycondensation and oxidative coupling are presented as the non-vinyl graft copolymerization methods. Then, the ring-opening graft copolymerization is described and the cases of the cyclic monomers \(\alpha\)-aminoacid \(N\)-carboxy anhydrides and \(\varepsilon\)-caprolactone are investigated. An extensive description of the “grafting onto” approach is provided. The preformed polymers discussed here for grafting onto chitin/chitosan include living poly(2-alkyl oxazolines), poly(ethylene glycol)s, block polyethers, poly(ethylene imine)s, poly(2-hydroxyalkanoate)s, polyurethanes, poly(dimethylsiloxane)s, and dendrimer-like hyperbranched polymers. Chitin/chitosan multiple modification including graft copolymerization is also investigated. Regioselective grafting using derivatives such as 6-iodo-, mercapto-, deoxy(thiosulphato)-chitins, and \(N\)-trichloroacetyl chitosan are described as suitable approaches to achieve chitin/chitosan graft copolymers with well-known structures.

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INTRODUCTION

Cellulose and chitin as biopolymers are the most abundant organic compounds in Nature and estimated to be at levels approaching $10^{11}$ tons annually [1]. Chitin has been a major structural component of animal exoskeleton since the Cambrian Period, more than 550 million years ago. The total amount of chitin harvestable without imbalancing the marine ecosystem is estimated to be $1.5 \times 10^{8}$ kg/year [2], mostly from the shells of crustaceans such as crab, shrimp and krill. Although cellulose has been studied extensively, only limited attention has been paid to chitin, principally from its biological properties [1,3]. Despite its huge annual production and easy accessibility, chitin still remains an unutilized biomass resource primarily because of its intractable bulk structure [1]. However, as Khor has stated [3], the 21st century can be the century of chitin taking a place as an extraordinary material, because chitin and its derivatives have exhibited high potential in a wide variety of fields including medical, pharmaceutical, cosmetics, bio-related science and technology, food industry, agriculture, and environmental protection [4-9]. Ravi Kumar has emphasized on the pharmaceutical applications of these biopolymers in his recent reviews [9-11]. Some reviews are published on fibre- and film-forming capabilities of chitin and chitosan [12,13]. In a very fascinating field i.e., gene therapy, chitosan and its appropriate derivatives are recently found to be excellent candidates for controlled gene delivery [14-18]. Overall, as exhibited in Figure 1, either scientific [3] or patent [7] literatures reveal a considerable growing in the field of chitin/chitosan science and technology from mid 1980s.

Chitin is structurally similar to cellulose, but it has acetamide groups at the C-2 positions instead of hydroxyl groups. So it is a nitrogen (amido/amino) containing polysaccharide, with repeating units of 2-acetamido/amino-2-deoxy-(1→4)-β-D-glucopyranose (Scheme I). In addition to its unique polysaccharide architecture, the presence of a little amino groups (5-15%) in chitin [4,19] is highly advantageous for providing distinctive biological functions and for conducting modification reactions [7,20,21]. Chitosan is the N-deacetylated derivative of chitin, though this N-deacetylation is almost never complete [20,21]. Actually, the names chitin and chitosan correspond to a family of polymers varying in the acetyl content.

![Figure 1. Annual number of chitin/chitosan related reports; (a) patents, 1966-2000 [7], (b) scientific research articles in the 1990s as obtained from ScienceDirect [3].](image)

![Scheme I. Structural repeating units of chitin (DD=5-15%) and its deacetylated product, chitosan (DD≥40%).](image)
Therefore, the degree of acetylation (DA) determines whether the biopolymer is chitin or chitosan. Chitosan is the term used for the considerably deacetylated chitin that is soluble in dilute acetic acid (degree of deacetylation, DD 70%) [1].

In spite of potential applications of chitin and chitosan, it is necessary to establish efficient appropriate modifications to explore fully the high potential of these biomacromolecules. Chemical modifications of chitin are generally difficult owing to the lack of solubility, and the reactions under heterogeneous conditions are accompanied by various problems such as the poor extent of reaction, difficulty in selective substitution, structural ambiguity of the products, and partial degradation due to severe reaction conditions. Therefore, with regard to developing advanced functions, much attention had been paid to modification of chitosan rather than chitin.

Chitin and chitosan have been modified via a variety of chemical modifications. Some authors have reviewed the methods [1,4] and Roberts have explained the modification reactions in his source-book Chitin Chemistry [20]. Of the various possible modifications (e.g., nitration, phosphorylation, sulphation, xanthation [14], acylation, hydroxyalkylation [4], Schiff’s base formation and alkylation [4,20-22]), graft copolymerization is expected to be one of the most promising approaches to a wide variety of molecular designs leading to novel types of hybrid materials, which are composed of bio- and synthetic-polymers [23]. This modification technique, as foreseen by Kurita [1], will likely find new applications in some fields including water treatment, metal cation adsorption, toiletries, medicine, agriculture, food processing and separation. This method has not been explored extensively, so that the Robertsí famous sourcebook comprises only less than one page on this topic. A literature review showed that, except a very short article published in 1996 by Kurita in the Polymeric Materials Encyclopedia [24], there is no review article on the graft polymerization onto chitin and chitosan.

During recent years, various research-teams as well as the author and coworkers [25-33] have focused remarkably on graft copolymerization as a versatile method of chitin and chitosan modification. This fascinating technique may be considered as an approach to achieve novel chitin/chitosan-based materials with improved properties including all the expected usefulness of these biomaterials [4-11]. As concluded in the present article, chitin will not be a biomaterial in waiting any more.

**GRAFT COPOLYMER SYNTHESIS**

A graft copolymer is a macromolecular chain with one or more species of block connected to the main chain as side chain(s) [34]. Thus, it can be described as having the general structure shown in Scheme II, where the main polymer backbone poly(A), commonly referred to as the trunk polymer, has branches of polymer chain poly(B) emanating from different points along its length. The common nomenclature used to describe this structure, where poly(A) is grafted with poly(B), is poly(A)-graft-poly(B), which can be further abbreviated as poly(A)-g-poly(B).

Grafting of synthetic polymer is a convenient...
method to add new properties to a natural polymer with minimum loss of the initial properties of the substrate. Chitin and chitosan possess aforesaid useful properties that render them interesting starting materials for the synthesis of graft copolymers. Most of the copolymers are prepared through graft polymerization of vinyl monomers onto the biopolymer backbone [31]. But this is not the only approach for synthesizing a grafted product. Because the chemistry of grafting vinyl monomers is quite different from that of grafting other monomers (or performed side chains), this section is divided into two subsections, the first dealing with grafting of vinyl monomers, the second reviewing other types of grafting methods.

**Vinyl Graft Copolymerization**

Grafting of polyvinylic and polyacrylic synthetic materials on the polysaccharides are mainly achieved by radical polymerization. Graft copolymers are prepared by first generating free radicals on the biopolymer backbone and then allowing these radicals to serve as macroradicals for the vinyl (or acrylic) monomer (Scheme II). Mino and Kaizerman firstly reported this approach in 1958 for graft copolymer preparation using a ceric ion redox initiating system [36]. Then, the chemistry and technology of the radical graft copolymerization technique [23,37] was developed especially in the case of cellulose [38] and starch [34,39]. Generally, free radical initiated graft copolymers have medium to high molecular weight branches that are infrequently spaced along the polysaccharide backbone [38]. The copolymerizations can also be initiated anionically by allowing monomer to react with an alkali-metal alkoxide derivative of polysaccharide. However, this method has not been progressed due to difficulty of the process and the low molecular weight of the grafted branches [39]. The properties of the resulting graft copolymers may be controlled widely by the characteristics of the side chains including molecular structures, length, number, and frequency.

One of the most important features of graft polymerization is unwanted formation of homopolymer, homopoly(B), that is not chemically bonded to the substrate poly(A). Homopolymer can result if the initiator used is one that produces free radicals in solution (in the presence of vinyl monomer B initiating homopolymerization) before creating the macroradicals. Once a grafted chain has been initiated and begins to propagate, chain transfer from the growing grafted chain end can occur with some species in the medium to yield free radicals that could initiate the growth of homopoly(B) chains [23].

To evaluate the efficiency of the graft copolymerization, the homopolymer is extracted with an appropriate solvent. Then, the homopolymer percentage (Hp%) and other various grafting compositional parameters are calculated. Although there are no unified definitions for calculating the parameters, the most frequently reported expressions for all kinds of graft copolymerizations are as follows [23,34,40]:

\[
\text{Graft yield (G\%)} = 100 \left( \frac{W_3 - W_0}{W_0} \right)
\]

\[
\text{Add-on (Ad\%)} = 100 \left( \frac{W_3 - W_0}{W_3} \right)
\]

\[
\text{Hp\%} = 100 \left( \frac{W_2 - W_3}{W_2} \right) \quad \text{or,}
\]

\[
\text{Hp\%} = 100 \left( \frac{W_4}{W_1} \right)
\]

where \(W_0, W_1, W_2, W_3\), and \(W_4\) designate the weight of the original substrate, monomer charged, total product (i.e., copolymer and homopolymer), pure graft copolymer, and homopolymer, respectively.

The various initiating systems employed to graft copolymerize different vinyl monomers onto chitin or chitosan can be categorized to two main classes, i.e. chemical initiation and radiation initiation that are investigated in the next sections.

**(a) Chemically-initiated Vinyl Polymerization**

Among the variety of chemical reagents reported for initiating the vinyl monomer graft copolymerization onto chitin/chitosan, ceric ion initiation and Fenton’s initiation are the most important systems. Cerium in its tetravalent state is a versatile oxidizing agent used most frequently in the graft copolymerization of vinyl monomers onto cellulose and starch [34-40]. It forms a redox pair with the anhydroglucose units of the polysaccharide to yield the macroradicals under slightly acidic conditions. As with cellulose and starch, the ceric ion has been a useful initiation method for graft copolymerizing chitin and chitosan with typical vinyl monomers [27-32] due to the similarities in the chemical structures of these polysaccharides.

The mechanism of initiation for chitosan is
believed to begin with a complex formation of Ce$^{4+}$ with the primary amine and the hydroxyl groups at the C-2 and C-3 positions, respectively. The radicals responsible for the initiation of grafted copolymer chains using vinyl monomer are produced from the complex dissociation. A general mechanism for the reaction proposed recently by the author and coworkers [28] is shown in Scheme III. At higher temperatures (e.g., 90°C), it is proposed that the imine moiety shown in Scheme III is further hydrolyzed in the aqueous acidic conditions to the corresponding aldehyde, whereby oxidation to an acyl radical gives another site capable of initiating a grafted polymer chain [23].

Moderate to excellent yields accompanied various amounts of homopolymer are reported. Table 1 provides the grafting conditions and compositional parameters of the synthetic polymer graft chitin/chitosan prepared using the ceric-saccharide initiating system. According to the recent study by the author and coworkers [27,28], acrylonitrile was optimally grafted copolymerized onto chitosan in a homogeneous phase while a very low level of homopolyacrylonitrile (2 %) was formed (the first row of Table 1). Based upon the study, the optimum conditions for achieving the maximum grafting were determined to be as: chitosan amount 0.20 g, acetic acid 2% w/w, reaction temperature 50°C; AN 1.60 g, ceric ammonium nitrate (CAN) concentration 0.006 M, time 2 h. The grafting efficiency was recognized to remain almost unchanged with the reaction time. The grafting-time independence can be attributed to a decrease in concentration for both initiator and monomer and also to a reduction of the number of sites on the chitosan backbone accessible for grafting as the reaction proceeds. Empirical rate of the polymerization showed a first-order dependence on the monomer concentration and a half-order dependence on the initiator concentration. An overall activation energy of 44.9 kJ/mol was determined for this graft polymerization reaction [28]. The hydrophobically modified chitosan exhibited higher thermal stability than chitosan itself [30].

Kim et al. [41] reported the ceric-induced graft copolymerization of N-isopropylacrylamide (NIPAM) onto chitosan at 25°C to prevent a high level of homopolymer formation (Table 1). The maximum grafting yield (48%) was obtained at 0.5 M of monomer concentration, 0.002 M of CAN initiator and 2 h of the reaction time. They found a decreased percent of grafting when the initiator concentration was higher than 0.002 M. The grafting loss was attributed to lower macroradical formation at the expense of producing more homopoly(NIPAM). The excessive CAN may also be consumed for oxidation of the polysaccharide backbone leading to decreased molecular weight of the graft copolymer product.

Vinyl acetate (VAc), a less reactive monomer than acrylates, was also recently grafted copolymerized onto chitosan by CAN in dispersion medium at 60°C [42,43]. With an addition of 0.5-7.5 g of chitosan based

---

Scheme III. General mechanism for ceric-initiated graft copolymerization of a typical vinyl monomer, acrylonitrile (AN), onto chitosan [28]. The opening of the pyranose ring shown above is very rarely occurred along a chitosan chain, so the initial structure of the trunk polymer is not actually destroyed.
on 50 g VAc, the monomer conversion was found to be between 70 and 80% after 2 h of reaction. The experimental results indicated that the chitosan macromolecules not only took part in the copolymerization, but also served as a surfactant providing the stability of the dispersion particles. The particulate membrane chitosan-g-PVAc formed after drying exhibited higher toughness and lower water-absorption compared to non-grafted chitosan. The copper ion adsorption by the poly(VAc) grafted chitosan was also studied [43].

The monomer 4-vinylpyridine (4VP) is another non-acrylic monomer graft polymerized onto chitosan [44] under homogeneous conditions. Percent grafting was increased with the amount of the monomer, showing a tendency to level off at a 4VP concentration of 0.53 M. As given in Table 1, maximum graft yield (331%) was achieved in lieu of a very high CAN consumption (0.087 M). According to the authors, their used system has a drawback since it requires the precipitation of the obtained product in a basic medium, resulting in the coprecipitation of the excess cerium salts that cannot be separated from the grafted product. The purified graft copolymers showed swellability behaviour in 1:1 and 1:2 acetic acid:ethanol solvent mixtures and insolubility in ethanol, dimethylformamide (DMF), dimethylsulphoxide (DMSO) and tetrahydrofuran (THF).

Acrylic and methacrylic acids were graft polymerized onto chitosan by Shantha et al. [45] to tailor drug carriers. They have reported an ambiguous procedure of polymerization without giving the CAN concentration used. Although they did not achieve a high grafting percent (Table 1), they utilized graft copolymers for preparing functionalized chitosan beads by a polymer dispersion technique. The drug sulphadiazine was entrapped in the microspheres and the in vitro drug release profiles were established in either simulated gastric and intestinal fluids. A sulphobetain methacrylic monomer, N,N-dimethyl-N-methacryloxyethyl-N-(3-sulphopropyl) ammonium, was recently reported to be graft polymerized onto chitosan by ceric ion initiation [46]. A maximum percentage of grafting about 50% was obtained under an optimized condition (0.5 g chitosan in 50 mL of 2 wt% acetic acid aqueous solution, CAN 0.0182 M, monomer 0.1434 M, 60°C, 2 h). Thermal properties of the graft copolymer were found to be slightly different from the original chitosan.

Chemically modified chitosan microspheres were synthesized by graft copolymerization of a bifunctional macromolecular monomer, poly(ethylene glycole)
diacrylate onto chitosan backbone using CAN [47].

The products were fully characterized by spectral, thermal and morphological techniques. Since the preparative reaction resulted in cross-linked networks, the grafting parameters could not be determined.

Regarding chitin, limited reports have been published about the ceric initiated grafting. Kurita et al. [48] have reported an efficient and reproducible procedure for graft copolymerization of acrylamide (AM) and acrylic acid (AA) onto powdery chitin. As a solvent, water proved to be superior to conventional nitric acid solution (except the reactions with a small amount of the initiator CAN). In spite of the heterogeneous conditions, under optimized conditions (0.10 g chitin, 10 mL water, 0.8-1.6 g CAN, 0.30-0.43 g monomer, 60°C, 2h), around 240% and 200% graft yields were achieved for AM and AA, respectively. The resulting graft copolymers showed improved affinity for solvents and hygroscopicity compared to the original chitin.

Acrylic acid was graft copolymerized onto chitin using CAN by other workers as well [49]. They achieved a low grafting (45%) under conventionally acidified (1 M nitric acid) reaction conditions (chitin 1.00 g, water 45 mL, CAN 0.00345 M, AA 0.959 M, 60°C, 1 h). The chitin-g-poly(AA) was used to study the effect of carboxylic group of the graft copolymer on the metal binding ability of calcium ions in aqueous medium as a function of pH, contact time and the metal concentration. The maximum adsorption of the graft copolymer and the original chitin were found to be 0.50 and 0.19 mmol Ca²⁺ g⁻¹, respectively.

Ren et al. [50] modified chitin via graft polymerization of methyl methacrylate (MMA) by CAN. They found the optimized grafting conditions to be: chitin 0.50 g, water 220 mL, nitric acid 0.0383 M, CAN 0.0425 M, monomer 0.0199 M, 40°C, 5 h. Grafting percentage of about 300% was obtained under the conditions. Solubility of the highly poly(MMA) grafted chitins in various organic solvents showed significant changes, and a gel-like mass swelling was resulted. Fine films could be made from the gel-like material especially in DMF or dimethylacetamide, when a small amount of lithium chloride was used. Complementary studies revealed that the amphiphilic comb-like chitin derivatives containing PMMA side chains were able to form stable monolayers with high collapse pressure [51].

Fenton’s reagent is another frequently used initiator for graft copolymerizing vinyl monomers onto chitin/chitosan [52,53]. The reagent involves a redox reaction between the ferrous ion and hydrogen peroxide, providing hydroxyl radicals (Scheme IV). These radicals are believed to be responsible for creating the macroradicals on the polysaccharide backbone, by means of hydrogen abstraction, that initiate the growth of grafted chains with various monomers. Although H₂O₂ alone could be an adequate initiator for the copolymerization, there are reasons why reducing agents such as Fe²⁺ are used for grafting onto chitosan. In addition to the higher yield of radical production at much lower temperatures via the redox reaction, the chelating properties of chitosan with metal ions tend to promote OH radical formation in the vicinity of the chitosan in order to increase macroradical yields rather than homopolymer formation initiation.

MMA was graft copolymerized onto chitosan with grafting percentages of 400-500% with homopolymer yields of around 20-30% [52]. Methyl acrylate (MA) has also been grafted with yields of 250-300% while homopoly(MA) was produced in the range of 15-20% [53].

\[
\begin{align*}
H_2O_2 & & + & & Fe^{2+} \rightarrow & & \cdot OH + OH^- + Fe^{3+} \\
\cdot OH & & + & & Fe^{2+} \rightarrow & & OH^- + Fe^{3+} \\
\cdot OH + H_2O_2 & & \rightarrow & & H_2O + \cdot OOH \\
\cdot OOH + H_2O_2 & & \rightarrow & & \cdot OH + O_2 + H_2O \\
\end{align*}
\]

**Scheme IV.** Mechanism for hydroxy radical formation by means of Fentons’s reagent in aqueous media.

\[
\begin{align*}
Fe^{2+} + S_2O_8^{2-} & \rightarrow Fe^{3+} + SO_4^{2-} + \cdot SO_4^- \\
\cdot SO_4^- + H_2O & \rightarrow HSO_4^- + \cdot OH \\
\cdot OH + Chitin-H & \rightarrow H_2O + Chitin \cdot \\
\cdot OH + Fe^{2+} & \rightarrow OH^- + Fe^{3+} \\
\cdot SO_4^- + Fe^{2+} & \rightarrow SO_4^{2-} + Fe^{3+} \\
\end{align*}
\]

**Scheme V.** Mechanism for macroradical formation on the backbone of chitin by means of ferrous ion-persulphate redox system in aqueous media.
Since chitin in less reactive than chitosan, its chelating properties may be enhanced through the addition of thiocarbonate sites along the chitin backbone via the xanthate process, i.e. treatment with concentrated aqueous sodium hydroxide and carbon disulphide [54]. After treating the chitin thiocarbonate derivative with ferrous ion, the complex \((\text{Chitin-O-CS}_2\text{S}_2\text{Fe})_2\) is formed. The decomposition of the complex leads to free Fe\(^{2+}\), which is believed to react with hydrogen peroxide as shown in Scheme IV to produce OH radicals that subsequently create chitin macroradicals upon hydrogen abstraction. Grafting percentages on the order of 80 and 40% were obtained with this process using acrylonitrile and acrylic acid, respectively. In both cases, homopolymer was obtained but not quantified.

As an alternative method for grafting, a variation of Fenton’s reagent has been investigated. Thus, potassium persulphate (KPS) and ferrous ammonium sulphate are combined in a redox reaction that ultimately produces hydroxyl radicals being able to form chitin macroradicals (Scheme V). MMA was graft polymerized onto chitin with this system at grafting percentages of 300-500%, where 40-50% of the monomer was homopolymerized [55]. Potassium persulphate alone was only able to achieve around 80-90% grafting while producing similar yields of homopoly(MMA).

When a non-acrylic monomer, i.e. \(N\)-vinyl pyrrolidone, was employed to graft copolymerize onto chitosan using KPS alone, graft yields of 200-300% were obtained accompanied by 10-20% homopolymer yields [56]. After the grafting, the solubility of chitosan was markedly reduced either in common organic solvents or in dilute organic or inorganic acids. However, the solubility of the grafted chitosan substantially improved after adsorption of copper ions, becoming completely soluble in dilute hydrochloric acid.

Chitosan has been subjected to graft copolymerization, comparatively with MA and MMA monomers using KPS alone and KPS coupled with various reducing agents [57]. The results are summarized in Table 2. No reference was made to a mechanism where the persulphate reacts specifically with the chitosan, so it is assumed the general mechanism proceeds as in Scheme V, where the other reducing agents (MnCl\(_2\), ammonium tartrate, etc.) may be substituted for the ferrous ion.

Hsu et al. [58] found that chitosan was degraded by KPS in aqueous media via a free-radical mechanism. This is an important point that should be taken into account in all the persulphate containing initiating systems. The same authors reported recently the synthesis of chitosan-modified PMMA by emulsion polymerization of different vinyl monomers onto chitosan. This table does not include the ceric- and Fenton's-based initiating systems.

Table 2. Various initiating systems employed for graft copolymerization of different vinyl monomers onto chitosan. This table does not include the ceric- and Fenton’s-based initiating systems.

<table>
<thead>
<tr>
<th>Monomer</th>
<th>Initiator system</th>
<th>G (%)</th>
<th>Hp (%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMA</td>
<td>KPS</td>
<td>268</td>
<td>37</td>
<td>57</td>
</tr>
<tr>
<td>MMA</td>
<td>KPS-FAS(^a)</td>
<td>300-350</td>
<td>40-50</td>
<td>55</td>
</tr>
<tr>
<td>MMA</td>
<td>KPS-Cu(_2)</td>
<td>497</td>
<td>29</td>
<td>57</td>
</tr>
<tr>
<td>MMA</td>
<td>KPS-Mn(_2)</td>
<td>489</td>
<td>16</td>
<td>57</td>
</tr>
<tr>
<td>MMA</td>
<td>KPS-AOX(^b)</td>
<td>397</td>
<td>29</td>
<td>57</td>
</tr>
<tr>
<td>MMA</td>
<td>KPS-ATA(^c)</td>
<td>388</td>
<td>31</td>
<td>57</td>
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<tr>
<td>MA</td>
<td>KPS</td>
<td>281</td>
<td>63</td>
<td>57</td>
</tr>
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<td>MA</td>
<td>KPS-FAS</td>
<td>80-90</td>
<td>40-50</td>
<td>55</td>
</tr>
<tr>
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<td>KPS-Cu(_2)</td>
<td>48</td>
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<td>57</td>
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<tr>
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<td>57</td>
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<td>KPS-AOX</td>
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<td>4</td>
<td>57</td>
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<td>40</td>
<td>57</td>
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<td>KPS</td>
<td>200-300</td>
<td>10-20</td>
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<tr>
<td>MMA</td>
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<td>276</td>
<td>-</td>
<td>60</td>
</tr>
<tr>
<td>AN</td>
<td>KPS</td>
<td>249</td>
<td>-</td>
<td>60</td>
</tr>
<tr>
<td>AM</td>
<td>KPS</td>
<td>220</td>
<td>-</td>
<td>61</td>
</tr>
<tr>
<td>AA</td>
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<td>52</td>
<td>-</td>
<td>62</td>
</tr>
<tr>
<td>AMPS</td>
<td>KPS</td>
<td>180</td>
<td>-</td>
<td>63</td>
</tr>
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<td>MA</td>
<td>DPIC(^d)</td>
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<td>64</td>
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<tr>
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<td>10</td>
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<td>MMA</td>
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<td>AN</td>
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<td>APS</td>
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<td>50</td>
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</tr>
<tr>
<td>MMA</td>
<td>(H_2)(_2)O(_2)</td>
<td>300</td>
<td>50</td>
<td>23</td>
</tr>
<tr>
<td>MMA</td>
<td>TBB(^e)</td>
<td>40</td>
<td>50</td>
<td>66</td>
</tr>
</tbody>
</table>

\(^{a}\) Ferrous ammonium sulphate, \(^{b}\) Ammonium oxalate, \(^{c}\) Ammonium tartrate, \(^{d}\) Potassium diperiodatocuprate (III), \(^{e}\) Tributyl borane.
tion [59]. They verified that chitosan played multiple roles in the emulsion. In addition to a surfactant role, KPS can appreciably be deactivated by low molecular weight chitosans produced by the persulphate-induced degradation. Therefore, long reaction times will not favour the formation of high copolymers when they are initiated by a persulphate system.

Most recently, KPS-initiated graft copolymerization of acrylonitrile (AN) and MMA onto chitosan was reported [60]. Maximum graft yield of chitosan-g-PAN (249%) was obtained with 0.12 M of AN and 0.00074 M of KPS at 65°C for 2 h for 1% chitosan solution. For chitosan-g-PMMA, 0.14 M of MMA at 65°C gave maximum grafting (276%). No residual monomers were found by HPLC in the graft copolymers. Thin membranous films could be prepared by thermopressing the modified chitosans.

Yazdani-Pedram et al. have recently studied the effect of reaction variables on KPS-initiated graft copolymerization of acrylamide onto chitosan in the presence of N,N'-methylenebisacrylamide (MBA) cross-linker [61]. They found the optimum reaction conditions (0.8 g chitosan, 50 mL acetic acid 2%, 2.4 g AM, 0.25% MBA, 0.002 M potassium persulphate, 60°C, 30 min.) to achieve high grafting percentage (~220%). Acrylic acid has also been graft copolymerized onto chitosan by the same research group [62]. They employed KPS-ferrous ammonium sulphate (FAS) redox initiating system to achieve low efficiency of grafting (52%) under optimized conditions (chitosan 0.3 g, water 50 mL, acrylic acid 2 mL, KPS 0.01 M, FAS 0.00006 M, 70°C, 2 h). All the grafted products were insoluble in water and in dilute acid solutions. The insolubility is the main difference between the graft copolymers and the chitosan itself which readily dissolves in slightly acidic media. The grafted chitosan samples, however, were swelled in these media. The swelling properties of the highly swollen poly(acrylic acid) grafted chitosan hydrogels were also studied [62].

Optimized synthesis of a graft copolymer based on chitosan and 2-acrylamido-2-methylpropane sulphonylic acid (AMPS) was reported by Najjar et al. [63] using KPS in homogeneous solution. The maximum percentage of grafting of ~180% was achieved under the optimum conditions (1% v/v acetic acid, 50°C reaction temperature, 10 min. chitosan-KPS mixing period, 0.37 mmol of KPS, and 28.96 mmol AMPS).

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Most recently, a novel redox system, Cu(III)-chitosan, was employed to initiate the graft copolymerization of MA onto chitosan in alkaline aqueous media [64]. A maximum grafting of around 650% was achieved under the optimum conditions concluded: 0.3 g chitosan (DD 0.82, mesh 60), 2.6 mL MA, pH 11.6 (by adding KOH solution), Cu(III) 0.00192 M, 35°C, 1 h. According to a proposal of the authors, a single electron transfer from -NH2 of chitosan to Cu(III) occurs to produce a radical-cation converted subsequently to -NH radical in alkaline medium. The -NH radical is thought to initiate the graft copolymerization (Scheme VI(a)). The trivalent copper, as the salt potassium diperiodatocuprate (III), was easily prepared from the cheap copper salt, CuSO4.5H2O. Since the grafting reaction can be carried out at a mild temperature of 35°C using a cheap initiator, the initiating system was recognized to be superior to conventional initiators [64].

A redox system based on a supernormal valence transition metal, i.e. Ni(IV)-chitosan, was recently reported in the Chemical Journal on Internet [65]. Thus, acrylonitrile was graft polymerized onto chitosan (MW 2-3*10^5, DD>0.82) using potassium diperiodatonicelate (IV). Under the optimized copolymeriza-
tion conditions (Ni(IV) 2.25 mM, monomer/chitosan wt. ratio 5.3, 32°C, 2 h) for treating 0.3 g of chitosan, a grafting percentage of 110% was achieved. Based on the FTIR spectral N-H zigzag bands and the previous reports, the authors verified the process involving a two-step single electron transfer mechanism for the initiation of the polymerization (Scheme VI(b)).

Azobisisobutyronitrile (AIBN), ammonium persulphate (APS), and hydrogen peroxide (H₂O₂) are commonly employed to graft copolymerize vinyl monomers onto chitin and chitosan [23,66]. Under heating (or irradiation), the first radicals produced by these systems occur from homolytic bond scissions of the initiator, whereby these radicals subsequently react with the monomer to initiate the polymerization. For typical graft copolymerization, these radicals provided by the initiator, in addition to reacting directly with vinyl monomer, abstract hydrogens from chitin or chitosan creating macroradicals that are capable of initiating a grafted chain with vinyl monomers. Vinyl acetate, AN, MA, and MMA are graft polymerized onto chitosan using this kind of initiation system. The typical results are summarized in Table 2.

Tributylborane (TBB) was also utilized for initiating the grafting onto chitosan. According to Kojima et al. [67], the alkylborane-initiated polymerizations of various vinyl monomers in the presence of oxygen occur by means of a free-radical mechanism, prompting the investigation of the initiator for the grafting of chitin with MMA. Based on the proposed mechanism, a solvated chitin-TBB complex produces radicals on the chitin backbone that in turn, initiate the graft polymerization. The system produces homopoly(MMA) value as high as 50% and low level of graft yield (about 40%).

(b) Radiation-initiated Vinyl Polymerization
Both high- and low-energy radiation may be used for graft copolymerization of vinyl monomers onto polysaccharides. The radiation-induced grafting onto celluloses has been discussed in the chapter 3 of the sourcebook of Hebeish and Guthrie [38].

Employing high-energy radiation (e.g., β, γ, X-ray) is an efficient basic method for initiating radical graft polymerization onto polysaccharides. The initiation method with the highest graft efficiency seems to be pre-irradiated of the polysaccharides with γ-radiation followed by the activated polysaccharide with vinyl monomers under suitable reaction conditions [35,38]. Grafting efficiency means in this connection not only a high number of grafted branches with high molecular weights, it means in particular a low level of homopolymer formed. Although the radiation-based grafting is cleaner and more efficient in this regard than chemical initiation methods, they are harder to handle under technical conditions [35]. This is why the number of researches on irradiation methods have been considerably smaller that that of the chemical methods.

Irradiation of γ-ray on powdery chitin initiates the graft polymerization of styrene, as in the case of cellulose, but the grafting percentage is low (i.e., 64%). Styrene was also graft copolymerized onto chitosan powders or films. Water was recognized to be essential for the both grafting reactions. The chitosan-g-poly-styrene adsorbed bromine better than chitosan, and the copolymer films showed less swelling and higher elongation in water than what the chitosan films did [1,68].

Pengfei et al. [69] recently reported the γ-radiation-induced graft copolymerization of styrene onto chitin and chitosan powder. The reaction was promoted in the presence of methanol, and atmospheric oxygen delayed the reaction but did not inhibit it completely. Molecular weight of the grafted polystyrene did not change obviously with the dose of radiation, but it was increased with the increase of the concentration of the monomer charged. The polydispersity index of the grafted chains was measured to be basically between 1 and 2.

Singh and Ray graft copolymerized 2-hydroxyethylmethacrylate (HEMA) onto chitosan films using ⁶⁰Co gamma radiation to improve their blood compatibility [70]. They found that the level of grafting could be controlled by the grafting conditions, namely solvent composition, monomer concentration, dose rate, and total dose. They achieved a maximum graft yield of 108% under the conditions of solvent water-methanol volume ratio 1:1, HEMA concentration 20 vol%, dose rate 90 rad/s and total dose 0.216 Mrad. The swelling of this PHEMA-grafted film in phosphate buffer (pH 7.4, 0.1 M) at 37°C was 58% compared to that of the original chitosan film, i.e. 110%.

Chitosan films have also been subjected to gamma radiation-induced graft copolymerization of the vinyl monomer N,N-diethylaminoethyl methacrylate [71].
The reaction variables affecting on the grafting percentage have been studied. The grafting of 70% is being achieved under the conditions of solvent water-methanol volume ratio 1:1, the monomer concentration 15 vol%, dose rate 90 rad/s and total dose 0.216 Mrad, and irradiation time 80 min. The grafted chitosan was fully characterized by swelling and tensile measurements, and FTIR, DSC, TGA and XRD methods. The degree of swelling, crystallinity, and tensile strength were decreased by 51, 43, and 37%, respectively, at a graft level 54%, whereas the modified films showed improved thermal stability [71].

Low energy photons may also initiate the polymerization of a vinyl or acrylic monomer if the irradiation is carried out in the presence of an activator (photosensitizer). Such a sensitizer must become active on exposure to the particular wavelength range of the incident radiation [38]. Irradiation with low energy radiation, i.e. visible or ultraviolet (UV) lights, usually in the presence of a photosensitizer such as benzophenone or azo compounds, is a rarely used method for grafting onto chitin/chitosan. UV-initiated graft copolymerization of MMA onto chitosan has been reported [23,72]. The grafting percentage was decreased in order: Photo-initiation (by the light of 253 nm) without a catalyst (G 300%, Hp 30-40%) > photoinduced method with photosensitizer AIBN (G 150%, Hp 60%) > photoinduced method with photosensitizer benzophenon (G 140%, Hp 40%).

Under the noncatalytic photoinduced initiation conditions (i.e., the irradiation of only chitosan, solvent and monomer), it was proposed that the amine group of chitosan be removed by the photolysis [23]. When a mixture of acrylonitrile and styrene was used, almost alternation copolymers were introduced [24].

Photo-induced initiation was also applied to the graft copolymerization of MMA onto chitin or oxychitin (oxidized chitin). A small amount of dimethylformamide reduced the induction period and increased either the grafting percentage or the apparent number of grafted chains. Non-catalytic photo-induced graft polymerization was smooth and obtained higher graft yield (G 150%, Hp 50%), compared to the grafting photo-sensitized with H₂O₂ (G 70%, Hp 50%) or AIBN (G 60%, Hp 90%) [23,72].

Some pre-designated chitin/chitosan derivatives have also been subjected to irradiation-induced graft copolymerization to yield indirectly the corresponding grafted products. They are explained in the next section entitled grafting onto pre-modified chitin/chitosan.

### Non-vinyl Graft Copolymerization

(a) **Graft Copolymerization via Polycondensation**

Condensation polymerization has not been widely used for preparing graft copolymers of polysaccharides usually due to susceptibility of the saccharide backbone to high temperature and harsh conditions of the typical polycondensation reactions.

However, lactic acid (LA) was successfully graft copolymerized onto chitosan through condensation polymerization of D,L-lactic acid in absence of a catalyst [73]. So, the bio-active and compatible polymer polylactic acid (PLA) is conjugated with the biopolymer chitosan through an amide linkage.

The degrees of substitution (DS) ranged from 6 to 18 were measured using both elemental analysis and salicylaldehyde methods. Degree of polymerization (DP) of the PLA side chains was determined, by H NMR analysis, to be in the range of 0.9-4.4. The graft copolymers were found to be physically cross-linked during the polymerization leading to pH-sensitive chitosan-PLA hydrogels. Water uptake of the hydrogels was investigated as a function of values of DP, DS, pH and salt concentration. The structural change and swelling mechanism of the hydrogels were also investigated [74]. A study on cytocompatible chitosan-g-PLA copolymer film has recently been reported [75]. The results showed that the cell growth rate on the film was obviously faster than chitosan itself.

(b) **Graft Copolymerization via Oxidative Coupling**

With a view to prepare conductive polymers, polyaniline was grafted onto chitosan [24]. On the treatment of chitosan in aqueous acetic acid solution with aniline in the presence of APS, polyaniline side chains were introduced at the amino groups. Chitosan-g-polyaniline was fabricated into films and fibres, but the properties varied according to the ratio of amino group to aniline in the grafting reaction. With a ratio of 1/1 to 1/5, the products were sturdy and flexible, while those with a 1/6 to 1/10 ratio were brittle. Optical microscopic observations indicated that the products prepared at a ratio below 1/5 were homogeneous, but those of 1/6 ratio had crystalline regions. The graft copolymers...
were deep blue and became dark green when treated with hydrochloric acid. The conductivity could be raised from less than 10^{-7} to 0.01 S/cm upon protonic doping with HCl [1,24].

**Cyclic Monomer Graft Copolymerization: Ring-opening Method**

In general, four groups of cyclic monomers have been mainly used for graft copolymerization onto polysaccharides: oxiranes (epoxides), lactons, α-aminoacid N-carboxy anhydrides (NCAs) and 2-alkyl oxazolines [35]. However, the various NCAs have been the main type of cyclic monomer used to graft onto chitin and chitosan.

Chitin-g-oligo(caprolactone) was recently reported to be synthesized via ring opening graft polymerization of ε-caprolactone (CL) to partially deacetylated chitin catalyzed by tin(II) 2-ethylhexanoate in the presence of water as a swelling agent [76]. Thus, N-substituted graft copolymer with 40 wt% oligo(CL) (average degree of polymerization ~4) was obtained by the reaction conditions: 0.20 g chitin (DD 0.51), catalyst 0.17 mol%, water 130 mol%, 100°C, 20 h. The structure of the copolymer was fully characterized by IR, NMR and XRD methods.

Partially deacetylated chitin have been grafted with D,L-alanine NCA, γ-methyl L-glutamate NCA, and L-alanine NCA [23,24,77]. NCA ring can undergo nucleophilic attack to open and polymerize with evolution of CO₂ to yield a polypeptide chain [78]. As shown in Scheme VII, the free amine of the deacetylated chitin is believed to initiate the graft copolymerization by means of attack upon carbonyl, ultimately creating the grafted chitin derivative. The advantages of this method are low level of homopolymer formation and the possibility of the side chain length control by the regulation of the NCA concentration under proper conditions. Degree of polymerization (DP), however, is not usually higher than 20 [23]. The resulting copolymers are new types of hybrid materials composed of both a polysaccharide and polypeptides.

Chitosan or water-soluble chitin failed to initiate these reactions in organic solvents due to low extents of swelling and rapid hydrolysis of NCAs [1]. A water-soluble chitin (50% deacetylated chitin), however, exhibited high reactivity in aqueous solution. It was treated with an ethyl acetate solution of γ-methyl L-glutamate NCA [24]. The ring-opening graft polymerization of the NCA proceeded smoothly at 0°C to give chitin-g-poly(γ-methyl L-glutamate). Although NCAs are very susceptible to hydrolysis, the grafting efficiency was surprisingly high, up to 91%, and no homopolymerization was observed. On alkaline hydrolysis, side chain ester groups were transformed into carboxylate groups. Graft copolymerization of NCAs onto partially deacetylated chitins is also possible in DMSO, but the grafting efficiency was moderate because of the heterogeneous reaction conditions [1]. Chitin-g-poly(γ-methyl L-glutamate) copolymers have shown varying degrees of solubility in common polar solvents depending on the side chain length [1,24].

Poly(L-alanine) side chains were also introduced to chitin in a similar manner [77]. The grafted poly(L-alanine) chains could be regarded as spacer arms having a terminal free amino group which can be used for further modifications. They were utilized to immobilize dihydronicotinamide groups (the active site of coenzyme NDAH) (Scheme VIII). The resulting bioconjugates were utilized for asymmetric reduction of a ketone while the peptide spacer arms regulated the
reduction process [78].

**Preformed Polymer Grafting: Grafting Onto Method**

Grafting with telechelic polymers provides an alternative method, commonly referred to as grafting onto, for synthesizing hybrid branched architectures [79]. Telechelic polymers have been defined as those containing one or more functional end groups that have the capacity for selective reaction to form bonds with another molecule [80]. Unlike the classic grafting techniques where the grafted chain is grown from the trunk polymer by the continual addition of monomer to

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**Scheme VIII.** Immobilization of dehydronicotinamide group (coenzyme NADH active site) on partially deacetylated chitin [77,78].

**Scheme IX.** General representation of the “grafting onto” method for modification of trunk polymer poly(A) with preformed reactive (telechelic) polymer, poly(B) [79]. Reaction of functional group x with y leads to a covalent linkage.

**Scheme X.** Synthesis of living poly(2-alkyl-2-oxazoline), PAO, followed by its use to graft onto partially deacetylated chitin [81].
the growing chain end, grafting onto connects a pre-formed polymer chains (poly(B)) and the trunk polymer (poly(A)) by covalently bonding the chain end of the poly(B) with a particular site on poly(A)'s backbone (Scheme IX).

Living poly(2-alkyl oxazolines) telechelic polymers have been grafted onto partially deacetylated chitins by Aoi et al. [81] in DMSO. In this solvent, the water-soluble chitin swells to some extent, thus the amino group was used to terminate the living polyoxazolines synthesized by cationic ring-opening polymerization of the corresponding oxazolines with methyl trifluoromethanesulphonate (Scheme X). Full degrees of N-substitution of available amine groups have been obtained with grafted chain DPs ranging from 8 to 33 units [82]. The number of side chains was controlled by the amount of living polymers employed. This method enabled the introduction of monodispersed side chains. The grafted derivatives were miscible with PVC to varying degrees [83,84]. They were soluble in water, DMF, and DMSO, and partially soluble in chloroform, acetonitrile, and methanol. Living poly(2-methyl-2-oxazoline) and poly(isobutylvinyl ether) cation was successfully terminated by surface amino groups on chitosan powder to give the corresponding polymer-grafted chitosan [85].

According to a pioneering report published in 1999, the same research group [86] synthesized chitin derivatives with well-defined block copolymer side chains. In fact, they reported the first example of introduction of living block copolymer to a polysaccharide. The graft copolymers having monodisperse amphiphilic poly(2-oxazoline) block copolymer side chains exhibited associative behaviour and complexation with hydrophobic substances depending on the chemical structure. Among the derivatives, chitin-graft-[poly(2-}

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Scheme XI. Approaches for PEGylating chitosan, an outstanding case of grafting of a preformed polymer onto chitosan. The chitosan-g-PEG graft copolymer is often referred to as “PEGylated chitosan”. mPEG= methoxyterminated PEG, PNP= paranitrophenyl, WSC= water-soluble carbodiimide, BtOH= hydroxybenzotriazole.
methyl-2-oxazoline)-block-poly(2-phenyl-2-oxazoline)] formed cylindrical aggregates (diameter 40 nm, length 80-200 nm) in aqueous solution.

Poly(ethylene glycol) (PEG) have appeared to be a very important synthetic macromolecule in bio-science and technology [87,88]. The term PEGylation is usually referred to a process involving the conjugation of PEG with a substrate. The conjugation of PEG to drugs, especially protein drugs, is well known to enhance the solubility and stability of the protein in solution, to alter bioavailability, pharmacokinetics, immunogenic properties, and biological activities, and also to protect it from recognition by the immune system, prolonging its circulation time and efficacy in vivo [89]. Several methods have been reported on the PEGylation of chitin/chitosan using PEGs with various terminal reactive groups (Scheme XI). Harris et al. [90] have reported the synthesis and characterization of various functional PEGs useful for PEGylation.

PEGylated chitosans may be especially suitable as carriers for delivery of anionic drugs, such as proteins, glycosaminoglycans, and DNA plasmides or oligonucleotides [89]. Methoxy PEG p-nitrophenol carbonate (MW 5000) was used to link PEG to chitosan through a urethane linkage [89]. The synthetic route including the reaction of the chitosan free amine groups with methoxy PEG p-nitrophenol carbonate is shown in Scheme XI. Grafting yields were 80-90% based on the weight of chitosan, where grafted derivatives were soluble in aqueous solutions at pH 6.5, contrary to highly deacetylated chitosan.

Methoxy PEG acyl carboxylate (Mn = 5000) was activated with a carbodiimide/hydroxybenzotriazole technique used in peptide synthesis, to subsequently acylate chitosan free amine groups [91]. The product PEGylated chitosan chains were obtained with degrees of N-substitution (DS) ranging from 0.02 to 0.55, where the copolymers having DS greater than 0.10 were observed to be water-soluble after ultrasonication. Other workers [92] reported another approach for immobilization of proteins with PEG tresylates and chitosan tresylates. They found that the tresylate reacts by an unexpected mechanism with this regard, however, to give a sulphonateamide linkage as the major product rather than simple N-alkylation. PEG tresylate also PEGylates the thiols [88], and this may sometimes be a disadvantage.

PEGylation of chitosan via reductive alkylation of the amine group of the chitosan was firstly reported in 1984 by Harris et al. [90]. This facile one-pot synthetic method includes the reaction of the -NH2 with -CHO of an aldehyde-terminated PEG to form a Schiff-base. The imine unsaturation (–CH=N–) is then reduced by sodium cyanoborohydride (NaBH3CN) to produce a stable N-PEGylated chitosan (Scheme XI). This approach was then employed by other workers to efficiently prepare the chitosan-PEG copolymeric hybrids [90,91,93]. Sugimoto et al. [92,93] optimized the process to obtain purified chitosan-PEG hybrids with DS values substantially higher than those reported by the pioneers (DS 0.74 vs. 0.06). They then treated the hybrids with acetic anhydride to prepare chitin-PEG hybrids. The solubility of the graft copolymers in water was reported to be dependent on the PEG molecular weight, the weight ratio of PEG in the hybrids, DS, and degree of acetylation [93]. The modification with the higher molecular weight PEG improved water-solubility of chitosan keeping the main skeleton intact. The bioactivities of the PEGylated chitosans were studied as well [94,95]. PEG side chains with very low molecular weight (i.e., oligo(ethylene glycol) pendant chains) were also prepared via the same way to achieve comb-shaped chitosan derivatives [96]. The tri- and tetra(ethylene glycol) monosubstituted derivatives were characterized by high affinity for organic solvents as well as water in sharp contrast to the original chitosan. They showed significant adsorption capacity toward metal cations. It should be pointed out that the above-described reductive amination is also a potentially useful method for conjugation of any NH2 containing drugs to PEG.

The reductive amination is also possible by using PEG propionaldehyde or PEG acetaldehyde [97] to provide a direct amine link to PEG with retention of basic properties. However, the ease of polymerization of the PEG acetaldehyde in preparation has presented problems in application of these methods [98]. It has also been found that air oxidation of PEG acetaldehyde occurs readily and low-temperature storage under an inert atmosphere is thus necessary [99]. Bentley et al. came over these problems using a simple and reliable method for preparation and use in reductive amination of PEG acetaldehyde hydrate generated in situ by hydrolysis of PEG acetaldehyde diethylacetal. They demonstrated the application of their approach in PEGylation of lysozyme and chitosan (Scheme XI) to...
form water-soluble methoxy PEG (mPEG) derivatives and PEG-chitosan hydrogels [99].

In a three-step synthetic approach for chitosan PEGylation reported by Ouchi et al. [91], the C-6 hydroxyl group of chitosan was firstly protected by means of triphenylmethylolation. The 6-O-triphenylmethyl chitosan derivative was then coupled with mPEG acid (Mₙ 5000) in the presence of a water-soluble carbodiimide and a hydroxybenzotriazole. The protected hydroxyl group was finally deprotected by treating with acetic acid solution to yield chitosan-g-PEG in which the PEG has been grafted onto chitosan via an amide linkage (Scheme XI). The degree of PEG introduction was estimated by colloidal titration to be 25%. The article has focused on the aggregation phenomenon of the PEG-grafted chitosan in aqueous solution [91].

To control the elastic modulus of chitosan for applying in resorbable small diameter vascular grafts, polyethylene glycol was grafted onto chitosan using succinimidyl-propionate-PEG to produce PEG-chitosan graft copolymer [100] (Scheme XI). The grafting of PEG chains increased the distance between the polymer chains, thus reducing the crystallinity and resulting in a lower elastic modulus.

Triblock polyethers PEO-PPO-PEO, known as Pluronic polyols or poloxamers, were grafted onto chitosan and poly(acrylic acid) by A.S. Hoffman et al. [101]. They first activated one OH end group of the polyol with p-nitrophenyl (pNP) chloroformate and then conjugated directly with chitosan (MW 750000, DD 0.8) (Scheme XII) at various pHs. Dilute solutions (2-3 wt%) of the hybrid product, chitosan-g-[poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide)], formed gels upon warming from 4 to 34°C. The viscosity buildup increased significantly with the degree of grafting and decreases sharply with increasing shear rates.

Poly(ethylene imine) (PEI), a branched or linear commercially available polyamine, has also been grafted onto chitosan and poly(acrylic acid) by Yalpani et al. [106]. Thus, the chitosan solution in dilute acetic acid was treated with reduced molecular weight PHB (in the presence of dimethylsulphoxide at ambient temperature for 1-5 days) to afford the corresponding amide conjugates (Scheme XII). The degree of substitution of the chitosan amine functions in the bioconjugate was very low (DS 0.02-0.03), varying insignificantly over the range of PHB-chitosan ratios examined. The molecular weight of the PHB branches was around 10000.

Most recently, the synthesis of a polyurethane-grafted chitosan was reported by Silva et al. [107]. They firstly prepared the urethane prepolymer by condensation reactions of PEG of two different molar masses and isophorone diisocyanate. In a DMF/acetic acid (1:1) medium, the NCO terminated prepolymer was then grafted with chitosan backbone through a urea linkage (Scheme XII). The DS values varied from 0.03 to 0.6 depending on the reaction conditions.

Poly(dimethylsiloxane) (PDMS)-grafted chitosan was prepared and characterized [108]. Thus, PDMS prepolymer was synthesized by ionic ring-opening polymerization of octamethylocyclotrisiloxane using n-butyl lithium. The tensile strength and elongation of
chitosan-g-PDMS copolymer were mostly constant regardless of the grafting percentage. While critical surface energy of chitosan is about 0.032 N/m, that of the copolymer was a little decreased to 0.025-0.029 N/m by grafting PDMS onto chitosan.

Epoxy-terminated PDMS was grafted onto chitosan using UV irradiation at room temperature without using a catalyst. The product was a pH-sensitive hydrogel without a chemical cross-linking occurrence. In fact, the PDMS substituents provided the basis for hydrophobic interactions that contribute the formation of the hydrogel network. The hydrogels exhibited high equilibrium water content in the range of 82-92% [109].

Chitosan miniemulsions were used for the synthesis of epoxy particles by polyaddition. As chitosan bears amine and alcohol functions, it can react with the epoxide and can be grafted onto the particles, which are obtained by polyaddition reaction. This turned out to be a convenient technique to modify or graft the water-soluble chitosan with water-insoluble reaction partners, thus resulting in new and not accessible chitosan derivatives [110]. Here, also other biodegradable polymer (i.e., a protein) was added for hybrid formation (Scheme XII). Finally, nanocapsules consisting of hybrid polyaddition polymers as shell and a hydropho-

Scheme XII. Various synthetic routes to chitosans conjugated with different macromolecular pendant groups through “grafting onto” method. PEI= polyethyleneimine, PHB= poly(3-hydroxybutyrate), PEO= poly(ethylene oxide), PPO= poly(propylene oxide), pNP= para-nitrophenyl, G-APG= Gluadin APG (a partially hydrolyzed wheat gluten protein, MW ~5000), PPG= poly(propylene glycol), BPA= bisphenol A residue, PDMS= poly(dimethyl siloxane), PEG= poly(ethylene glycol), IPDI= isophorone diisocyanate (3-isocyanatomethyl-3,5,5-trimethyl-cyclohexyl isocyanate).
bic part as core were elaborated. These biocompatible and biodegradable capsules promise applications in drug delivery.

In the course of the reaction for grafting a preformed polymer onto a substrate, the preformed polymer may be converted to another polymer with totally different, but desirable, properties. Most recently, Zohuriaan-Mehr and coworkers have achieved a one-step reaction to prepare lightly cross-linked poly(sodium acrylate-co-acrylamide) grafted chitosans through hydrolytic treatment of chitosan/polyacrylonitrile blends. According to FTIR spectroscopy, no cyanide

Scheme XIII. Chitosan modification via dendronization [112,113]. TEG= triethylene glycol, R=H.
functional group was detected in the grafted chitosan. This chitosan-based copolymeric hydrogel exhibited super-swelling properties and ampholytic behaviour [111].

Dendrimer-like hyperbranched polymers, a new class of topological macromolecules, have recently been grafted onto chitosan. Tsubokawa et al. [112] reported the surface modification of chitosan powder by grafting of hyperbranched dendritic polyamidoamine. They found that the polyamidoamine was propagated from the surface of chitosan by repetition of two processes: (1) Micheal addition of methyl acrylate to the surface amino groups and (2) amidation of the resulting esters with ethylenediamine to give polyamidoamine dendrimer grafted chitosan powder (Scheme XIII).

Sashiwa et al. [113] synthesized a dendronized chitosan-sialic acid hybrid using convergent grafting of pre-assembled dendrons built on gallic acid and tri(ethylene glycol) (TEG) backbone. Thus, sialic acid dendrons bearing a focal aldehyde end group were synthesized by a reiterative amide bond strategy. Polyamine-ending trivalent (1<sup>st</sup> generation: G1) and nona-valent (2<sup>nd</sup> generation: G2) dendrons having gallic acid as branching unit and TEG as spacer arm were prepared and initially attached to a sialic acid p-phenylisothiocyanate derivative. The focal aldehyde sialodendrons were then convergently grafted onto chitosan by reductive amination in 76-80% yields. The DS of the sialodendrimer in the hybrid were 0.13 (G1) and 0.06 (G2).

The water solubility of these novel hybrids was further improved by N-succinylation of the remaining amine functionality.

During recent years, Sashiwa and Aiba (from the Green Biotechnology Research Group of The Special Division for Human Life Technology, National Inst. Adv. Ind. Sci. Tech., Osaka, Japan) have started extensive research on chitin and chitosan with collaboration of other academic/industrial centers. They often publish their results as original papers under continued titles: studies on chitin and chitosan and chemical modification of chitosan. Several articles among these series (including the parts 3, 6, and 8-11) have been focused on chitosan-dendrimer hybrids (CDHs). In the sixteenth part of the latter title, for example, the synthesis of polypropyleneimine dendrimer-chitosan hybrid has been reported [114]. The hybrids were prepared in 80-90% yield and DS of 0.11 (G1), 0.042 (G3), and 0.037 (G4). CDHs having carboxyl, ester, and PEG and various generations were also prepared using dendrimer acetel by reductive N-alkylation. The synthetic procedure could be accomplished by one-step reaction without organic solvent [115]. The DS of the dendrimers was 0.13-0.18. Perfectly or partially water-soluble CDHs could be obtained. Good biodegradation was observed in these hybrids.

Multiple Modification of Chitin/Chitosan

There are some cases in which: (i) the reactivity of chitin/chitosan itself is insufficient to participate in the desired reaction, (ii) the modified chitin/chitosan does not possess the desired properties or (iii) some site(s) of chitin/chitosan must be protected (and finally deprotected) to sustain during the modification reaction(s). In such instants, there are two general approaches via chemical modification when the graft polymerization is a certain pathway to achieve desired characteristics: (a) in-situ- and/or post-treatment of the graft copolymer, (b) graft copolymerization onto a previously modified chitin/chitosan. So, the products may generally be referred to as multiply modified materials.

(a) Further Modification of the Graft Copolymers

Recently, a hydrophobically modified chitosan, chitosan-g-polyacrylonitrile copolymer prepared through a ceric-induced graft polymerization [28,29], was post-treated under alkaline conditions to achieve novel polyampholytic smart superabsorbing hydrogels with pH-reversibility and on-off switching behaviour [30,31]. The superabsorbsents so called as H-chitoPAN, showed low salt sensitivity when the add-on value of the initial chitosan-polyacrylonitrile graft coplymer was low [31]. The both modified chitosans exhibited enhanced thermal stability over chitosan itself [30]. According to the authors, the sharp pH-responsiveness behaviour of the hydrogels is expected to be a promising factor of their possible application in many technologies such as controlled delivery of bioactive agents.

Another case is an in situ modification of poly(NIPAM) grafted chitosan in the course of the synthetic reaction. When the grafting reaction was conducted in the presence of glutaraldehyde (GA) as a cross-linker, thermo- and pH-sensitive interpenetrating polymer network (IPN) hydrogels were obtained. The
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hydrogels were comparatively studied with some chitosan/poly(NIPAM) blend IPHs with the same composition ratios [41]. The equilibrium water content of all the IPN samples dropped sharply at pH 6 and temperature higher than 30°C. Therefore, the IPN hydrogels exhibited swelling/deswelling changes in response to external stimuli such as pH and temperature were recognized to be useful as modulation systems in biomedical fields.

A similar approach was reported recently by Don et al. [43] in the case of graft copolymerization of VAc onto chitosan in the presence of GA. They obtained particulate membranes of chitosan-g-PVAc formed after drying the suspension mixture of the polymerization reaction [42]. The membranes were then subjected to copper ion adsorption experiments. The mechanical strength of the wet membranes was improved by the in situ cross-linking with GA. The copper ion adsorption quantity of the membranes, however, was disfavoured by the strengthening [43].

Recently, microspheres of chitosan-g-polyacrylamide cross-linked with GA were prepared to use for encapsulating indomethacin, a nonsteroidal anti-inflammatory drug [116]. The microspheres were characterized for drug entrapment efficiency, particle size, and water transport into the polymeric matrix as well as for the drug-release kinetics.

(b) Grafting onto Pre-modified Chitin/Chitosan

A few chitosan derivatives are reported to be graft copolymerized with some vinyl monomers via the conventional vinyl graft copolymerization. Hydroxypropyl chitosan was prepared, characterized, and subjected to graft copolymerization with methacrylic acid (MAA) using APS as an initiator [117]. The same monomer was recently graft copolymerized onto carboxymethyl chitosan (CMCTS) by APS. An optimized grafting conditions (for CMCTS 8 g/L) was reported to be APS 8 mmol/L, MAA 2.4 mol/L, 60-70°C, 2h. Grafting percentage as high as 1500% was reported under the optimal conditions [118].

Sodium salts of acrylic acid (AA) and MAA were graft polymerized onto carboxymethyl chitosan using APS initiator [119]. Under a tentative conditions (CMCTS 0.02 g, monomer 1.2 M, APS 0.4 mM, 70°C, 2h), grafting percentages 875 and 933% were achieved for CMCTS-g-PAA and CMCTS-g-PMAA, respectively.

\[
\text{Scheme XIV. Thiocarbonation-bromate redox initiation [121] of graft polymerization of a vinyl monomer onto chitosan (X=NH or O).}
\]

\[
\text{Chit-XH + NaOH} \rightarrow \text{Chit-X} + \text{NaOH} + \text{H}_2\text{O}
\]

\[
\text{Chit-X}+\text{NaOH} + \text{CS}_2 \rightarrow \text{Chit-XCSS} + \text{NaOH}
\]

\[
6\text{Chit-XCSS} + \text{BrO}_3 \rightarrow 6\text{Chit-XCSS} + \text{Br}^- + 3\text{H}_2\text{O}
\]

\[
\text{Chit-XCSS} \rightarrow \text{Chit-X} + \text{CS}_2
\]

\[
\text{R}\text{C} = \text{O} \xrightarrow{\text{Grafting}} \text{R}\text{C} = \text{O}
\]

As mentioned before, since chitin is less reactive than chitosan, its reactivity may be enhanced through the addition of thiocarbonate sites along the chitin backbone via the xanthate process, i.e. treatment with concentrated aqueous sodium hydroxide and carbon disulphide [54]. After treating the chitin thiocarbonate derivative with ferrous ion, the complex chitin xanthate-Fe(II) is formed. The decomposition of the complex leads to free Fe(II), which is believed to react with hydrogen peroxide as shown in Scheme IV to produce OH radicals that subsequently create chitin macroradicals upon hydrogen abstraction. Grafting percentages on the order of 80 and 40% were obtained with this process using acrylonitrile and acrylic acid, respectively.

Chitosan was graft copolymerized with hydroxyethyl methacrylate (HEMA) via a thiocarbonated chitosan-potassium bromate redox initiation approach [121] (Scheme XIV). For a chitosan thiocarbonated under the optimized conditions NaOH 1%, CS₂ 2%, material-to-liquor (M/L) ratio 1:20, 30°C, and 1 h, total conversion of the monomer ranged up to 75% and a maximum grafting of 38% was achieved under the...
optimized grafting conditions ($\text{KBrO}_3$ 0.6 mmol/g chitosan, formic acid 2 g/g chitosan, HEMA 75%, M/L ratio 1:30, 40°C, 2 h). The polymerization composite product (including the graft copolymer, homopoly (HEMA) and unreacted chitosan) was easily cast as films which were featureless in the scanning electron

Scheme XV. Macromolecular architecture of graft copolymeric chitins with well-known structures from tosyl-, iodo-, mercapto-, and deoxy(thiosulphato)-chitin derivatives [122-126]. Ac= acetyl, Me= methyl, Ph= phenyl, DMSO= dimethylsulfoxide.
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Most of the vinyl graft copolymerizations (e.g., the above case, pesulphate- and ceric-initiating systems) are comprised a simple manner, but the initiating site and hence the structures of the resulting copolymers are not well-defined. Kurita et al. showed that with iodo-, tosyl-, and mercapto-chitin derivatives, graft copolymers having well-defined structures could be prepared, because it would produce initiating species only at the position carrying the certain functional group [1, 122-125].

Tosylation (treatment with excess p-toluene sulphonyl chloride) was carried out on alkali chitin. 6-Tosylated chitins were then treated with sodium iodide in DMSO. The reaction proceeded smoothly to give 6-iodo-chitins that exhibited good solubility in the solvent [122]. On addition of a Lewis acid such as SnCl4 to iodo-chitin in nitrobenzene, reactive carbenium species were formed, and styrene was efficiently graft copolymerized by a cationic mechanism in a swollen state (Scheme XV). The grafting percentage (G%) was reported to be up to 800%. The Mn and PDI of the grafted polystyrene were measured to be 58000 and 1.5, respectively. Irradiation of UV light on iodo-chitin in DMSO solutions gave rise to the homolysis of the C-I bonds to form carbon free radicals, and styrene was graft copolymerized by a radical mechanism. The grafting percentage is low, however, no appreciable amount of homopolymer was detected [1].

6-Mercapto-chitin is another candidate for the macroradical initiator to prepare well-defined graft copolymers. Although mercapto-chitin is insoluble, it is expected to efficiently graft copolymerization owing to the presence of the readily dissociating mercapto groups and to swelling in organic solvents. Actually, styrene was graft copolymerized onto chitin efficiently in DMSO at 80°C, and the resulting G% reached almost 1000 [123]. The M_n and PDI of the grafted polystyrene were measured to be 974000 and 2.62, respectively. The G% and M_n values indicated that 4% of the mercapto groups were used for the graft copolymerization, and a polystyrene chain was attached on average to every 45 pyranose units. MMA was also graft polymerized onto mercapto-chitin under similar conditions to achieve chitin-g-PMMA copolymers [124] (Scheme XV). The G% value was enhanced with the amount of the monomer and reached above 1200 under appropriate conditions. The resulting graft copolymers exhibited remarkable affinity for various common organic solvents.

6-Tosyl-chitin and iodo-chitin can also serve as polymeric initiators for ring-opening polymerization of 2-methyl-oxazolines to give poly(N-acetylethyleneimine)s in dimethylacetamide solution at 80°C [125] (Scheme XV). Tosyl-chitin was recognized to be more suitable than iodo-chitin judging from the G% values [1, 125]. M_n of the side chains isolated from the copolymer of 160% grafting was 2700, indicating that 18% of the tosylate groups were actually utilized for initiating the graft copolymerization.

6-Deoxy(thiosulphato)chitin (S_2O_3-chitin, DS 0.49) was synthesized and introduced as a precursor for non-catalytic photo-induced graft copolymerization of MMA, AN, AA, and AM [126] (Scheme XV). Chitin was first tosylated and subsequently transformed into S_2O_3-chitin. UV irradiation (at a fixed temperature 50°C) easily proceeded the polymerization. The photolysis of the S_2O_3 groups (confirmed by IR spectra) carried only in quartz, not in a Pyrex tube. The monomer MMA and AN showed good activities. In the case of MMA, under optimized conditions (S_2O_3-chitin 0.15 g, water 10 mL, MMA 2 mL, 1h), G% value reached around 600.

Chitosan powder was grafted with methyl acrylate (MA) utilizing the trichloroacetyl/Mn_2(CO)_10 photoinitiating system by Jenkins and Hudson [127]. First, highly deacetylated chitosan was N-trichloroacetylated by trichloroacetic anhydride. The trichloroacetyl chitosan powder was then graft copolymerized heterogeneously with MA using the manganese carbonyl co-initiator photoactivated with 436 nm light at room temperature (Scheme XVI). Graft percentages greater than
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600% were obtained, while 20-30% of the poly(MA) formed was homopolymer.

In a multi-step synthetic approach for PEGylating chitosan [91], the C-6 hydroxyl group of chitosan was firstly protected by means of triphenylmethylation. The 6-O-triphenylmethyl chitosan derivative was then coupled with mPEG acid (Mn 5000) in the presence of a water-soluble carbodiimide and a hydroxybenzotriazole. The protected hydroxyl group was finally deprotected by treating with acetic acid solution to yield chitosan-g-PEG in which the PEG has been grafted onto chitosan via an amide linkage (Scheme XI). The degree of PEG introduction was estimated by colloidal titration to be 25%.

Unlike the most of approaches to PEGylate chitosan leading to N-substituted chitosan-PEG graft copolymer as illustrated in Scheme XI, most recently, chitosan-O-PEG graft copolymers were synthesized via etherification of N-phthaloyl chitosan by PEG monomethyl ether (mPEG) iodide in DMF in the presence of silver oxide, and finally, deprotection of the N-phthaloylated chitosan amino group [128]. Varying the ratio of mPEG iodide to chitosan, different degree of O-substitution of mPEG to monosaccharide residue of chitosan (5-197%) was obtained. The graft copolymers were soluble in water and aqueous solutions of wide pH range. Reduced viscosity of aqueous solutions of the copolymers was extremely low and similar to that of mPEG 2000.

Ohya et al. [129] reported a novel 6-step synthetic strategy (Scheme XVII) to tailor a new chitosan-g-polystyrene with well-known structure and amphiphilic...
properties. Thus, the amino group of chitosan was first-
ly protected by phthalic anhydride. The primary alco-
hol group of the N-phthaloylchitosan was protected by
tritylchloride. 6-O-tritylchitosan was then prepared
using hydrazine-deprotection of the amino group. 6-O-
tritylchitosan was coupled with 4-4i-azobis(4-cyanoval-
eric acid) (ACVA). Graft polymerization of styrene
onto the 6-O-tritylchitosan was carried out in DMF
employing the pendant ACVA moiety as an initiator.
After deprotection of trityl groups of the polymeriza-
tion products, chitosan-g-polystyrene graft copolymer
was obtained. Under the reported conditions (6-O-
tritylchitosan (DS 24 mol%) 0.2 g, styrene-to-ACVA
mole ratio 810, 60°C, 8 h), the weight percentage and

Scheme XVIII. The synthetic pathway to a novel gene delivery agent; galatosylated chitosan-graft-poly(vinyl pyrrolidone) [133]. Ac= acetyl, EDC= 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride, NHS= N-hydroxysuccinimide.
M\textsubscript{n} of polystyrene content of the copolymer was measured to be around 96% and 51000, respectively. The graft copolymer showed a micro-phase separated morphology.

In another approach to modification, a polymerizable group is firstly introduced to the chitosan backbone and the reactive derivative is then (co)polymerized. For example, treatment of chitosan with maleic anhydride led to O- and N-maleinated chitosan. This fully substituted derivative was then copolymerized with acrylamide by APS initiator to yield cross-linked copolymers. The products were characterized by remarkable swelling in water with a volume increase of 20-150 times [130]. Using NIPAM, a similar strategy was followed to prepare pH/temperature-sensitive hydrogels [131].

Most recently, Tanodekaew et al. [132] reported the preparation of acrylic grafted chitin for wound dressing application. Acrylic acid (AA) was first linked to chitin via esterification (H\textsubscript{2}SO\textsubscript{4}, 70°C, 1 h). The acrylic double bonds acted as the active grafting sites on the substrate that was further polymerized with AA monomer (KPS, 65°C, 4 h) to form a network structure. The swelling behaviour and gel strength were found to depend upon the monomer feed content. Chitin-poly(AA) 1:4 yielded optimal swelling and gel strength. The overall results of the cellular behaviour on the modified chitin film suggested that the material has a potential for biomedical applications particularly as temporary skin substitute.

In the field of gene delivery, a galactosylated chitosan-g-poly(vinyl pyrrolidone) was recently prepared to employ as hepatocyte-targeting DNA carrier [133]. Chitosan was coupled with lactobionic acid via an active ester intermediate using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC). Through a grafting onto method, a preformed synthetic water-soluble polymer, i.e. monocarboxylic terminated poly(vinyl pyrrolidone) (PVP), was then chemically conjugated with galactosylated chitosan (GC) utilizing EDC and N-hydroxysuccinimide (NHS) as an activator (Scheme XVIII). The complex formation of GC-g-PVP/DNA complexes was confirmed by agarose gel electrophoresis. The morphology of the complex that observed by atomic force microscopy had a compact and spherical shape, around 40 nm particle sizes at a charge ratio of 3. The DNA-binding property of the graft copolymer mainly depended on the molecular weight of chitosan and composition of the synthetic part of the bioconjugate.

**CONCLUSION**

The science and technology of chitin and chitosan are advancing quite rapidly as a result of expanding interest in these biopolymers, which have unique characteristics. With regard to developing advanced functions, much attention has been paid to modification of chitin and chitosan. Graft copolymerization is considered to be one of the most promising approaches to a wide variety of molecular designs leading to novel type of tailored hybrid materials.

The present review deals with the chemistry of modification of chitin and chitosan via graft copolymerization with an emphasis on synthetic approaches. The article includes the majority of published papers in the field. Overall, the main concluding remarks may be summarized as follows:

- Direct grafting of vinyl monomers onto the substrate via radical copolymerization, being a simple and useful method of modification, have classically been plagued by a lack of control over the mechanism. Here, there are many different reactions occurring simultaneously, namely, initiation, propagation, etc. This complicated reaction system results in ill-defined structures and non-desired homopolymer. Major efforts to reduce homopolymer formation have been attempted; however, it is still generated more or less.

- Graft copolymerization of vinyl monomers onto the substrate activated by high-energy radiation (pre-irradiation technique) can solve the homopolymer formation problem in some extent.

- Living polymerization systems may be considered as a good solution of the homopolymer problem, as mentioned in the case of grafting of living poly(2-alkyl oxazolines) or living poly(isobutylvinyl ether) onto partially deactylated chitin.

- Grafting onto special derivatives of the biopolymers (e.g. tosyl-, iodo- or mercapto-chitin) is a certain approach to achieve graft copolymers with well-defined structures.

- By employing the grafting onto method, a high level of control on the macromolecular structure is pos-
sible without suffering from the above mentioned problems. This multi-step approach, however, is time consuming and cost non-effective, especially when a certain functional group of the trunk polymer is necessary to be protected (and then deprotected).

- While the unique structure and properties of chitosan (e.g., biological and cationic polymer characteristics) are mainly originated from its free amino group, the most modification strategy lead to N-substituted chitosan copolymers, in which the majority of the amino groups are blocked. Therefore, retaining these functional groups is an important challenge for preserving the intrinsic properties of the biopolymer in the hybrid material. Protection of the NH₂ group, usually as phthaloyl group, grafting treatment(s), and deprotection of the amino groups may be considered as an appropriate sequence result in desirable regioselectivity.

- Chitosan and partially deacetylated chitin have been more frequently subjected to the grafting rather than chitin itself. The reason is mainly related to insolubility of natural chitin in most of possible reaction media. Therefore, the intractability of chitin has yet remained as a challenge for the researchers who insist to modify chitin. Anyhow, it should be pointed out that the harsher the reaction conditions, the higher the polysaccharide molecular weight loss will be.

This contribution is also intended to stimulate further research on chitin and chitosan modification in order to use these precious renewable biomaterials instead of the fossil-based materials used in bio-science and technology. Fast-growing academic/industrial activities in this regard, as obviously exhibited in Figure 1, ensures that chitin will not be a biomaterial in waiting any more.

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