Chitosan Amphiphilic Derivatives. Chemistry and Applications

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Abstract: Chitosan is a natural polymer composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit).

It has been described as a non-toxic, biodegradable and biocompatible polymer with very interesting biological properties, such as permeation-enhancing and mucoadhesive properties, anticoagulant and antimicrobial activity and so on.

Chitosan has been used in several areas such as biomedical, pharmaceutical and biotechnological fields as well as in the food industry.

Recently, there has been a growing interest in the modification of chitosan to improve its solubility in physiological conditions, to introduce new applications or to improve chitosan biological properties.

Research and development on a variety of amphiphilic copolymers containing hydrophobic and hydrophilic segments, have been very active due to their spontaneous self-assembly behaviour in aqueous media These smart transitions often lead to diverse functional compartment structures like micelles, vesicles and gels, which represent promising applications in the field of biotechnology and pharmaceutics.

The aim of the present paper is to review the latest advances in the synthesis of chitosan amphiphilic derivatives with a special emphasis in their applications.

1. INTRODUCTION

Chitin and chitosan are described as a family of linear polysaccharides consisting of varying amounts of β (1→4) linked residues of N-acetyl-2 amino-2-deoxy-D-glucose (glucosamine) and 2-amino-2-deoxy-D-glucose (N-acetyl-glucosamine) residues (Fig. (**1**)). Chitin is the second most abundant natural polymer in nature after cellulose and it is found in the structure of a wide number of invertebrates (crustaceans' exoskeleton, insects' cuticles) and the cell walls of fungi, among others. On the other hand, chitosan only occurs naturally in some fungi (*Mucoraceae)*. Commercial chitosan samples are typically prepared by chemical deacetylation of chitin from crustacean sources under alkaline conditions. Chitosan deacetylation is rarely conducted to full completion and therefore chitosan polymeric chains are generally described as a copolymeric structure comprised of D-glucosamine along with N-acetyl residues. Moreover, chitosan is also prepared with a wide range of molecular weights, from chitosan oligomers to high molecular weight chitosan samples [1]. Chitosan is a polycation whose charge density depends on the degree of acetylation and pH. This macromolecule can dissolve in diluted aqueous acidic solvents due to the protonation of $–NH_2$ groups at the C2 position. Chitosan oligomers are also soluble under physiological conditions. In acidic conditions, even fully protonated chitosan tends to form aggregates as a result of hydrogen bonds and hydrophobic interactions. This hydrophobic behaviour is based on the presence of both the main polysaccharide backbone and the N-acetyl groups at C2 position [2]. Chitosan is a non-toxic, biodegradable and biocompatible polymer, which presents very interesting biological properties as shown in Table **1** [3-17]. It is currently being explored intensively for its application in several fields such as pharmacy, biomedicine, agriculture, food industry and biotechnology (Table **2**) [18-62]. However, since the vast majority of chitosan samples are only soluble in diluted acid solutions due to the molecular weight of this polymer,

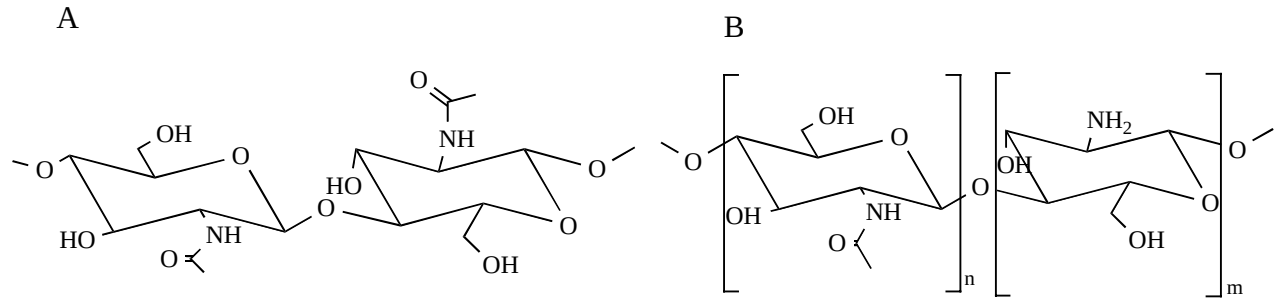

Fig. (1). A) Chemical structure of chitin (100% acetylation degree) and **B**) chemical structure of chitosan (where m>60).

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Table 1. Chitosan Biological Properties [3-17]

Property
Non-toxicity
Biodegradability
Biocompatibility
Citocompatibility
Mucoadhesion
Haemostatic action
Analgesic action
Adsorption enhancer
Antimicrobial activity
Anticholesterolemic activity
Antioxidant activity
Anti-inflammatory action
Angiogenesis stimulation
Granulation and scar formation
Macrophage activation

its applications are limited in some fields such as biomedicine, cosmetics, pharmacy or food industry.

In order to improve chitosan solubility in physiological media, to improve chitosan biological properties and widen chitosan applications, chitosan derivatives have been synthesised.

Among chitosan derivatives, amphiphilic chitosan derivatives are a type of molecules which have intensively been studied due to their interesting behaviour. However, these chitosan derivatives have not yet been reviewed in detail. The aim of this paper is to present a state-of-the-art study of the main methods that have been used to prepare chitosan amphiphilic derivatives in the last five years. A new method "RAFT polymerisation" is reviewed for the first time. A revision of the application of these molecules in different fields is presented, focusing on those applications that are not usually reviewed such as technological applications. Our group has recently reviewed the effect of the physico-chemical properties of chitosan on its biological properties and applications [63]. In a similar way, the effect of chitosan physico-chemical properties (deacetylation degree (DD) and molecular weight (Mw)) on the chemistry and applications of these derivatives is discussed.

2. CHITOSAN CHEMISTRY

As can be seen in Fig. (**1B**), chitosan has a primary amino group, and a primary and secondary free hydroxyl groups. The strong functionality of chitosan (two hydroxyl groups (C3, C6) and one primary amine group (C2) per-repeat unit) gives it a considerable opportunity of chemical modification. Several reviews covering the chemistry of chitosan have been recently published [64, 65]. A summary of chitosan chemistry is shown in Table **3**. Chitosan can be depolymerised to reduce its molecular weight and viscosity, improving also its solubility in aqueous media. Chitosan solubility

Table 2. Some Chitosan Applications

Biomedicine/pharmacy [19-35]	Wound dressing Drug delivery systems (oral, parenteral, nasal, ocular, pulmonary, transdermal administration) Gene delivery Tissue engineering Orthopaedic, periodontal applications Excipient Radiopharmaceuticals
Cosmetics [36-40]	Maintain skin moisture Protect epidermis Acne treatment Reduce static electricity in hair Tone skin
Technology [41-51]	Biocatalyst (enzyme/cell immobilisation) Water engineering Molecular Imprinting Metal reduction, nanoparticle stabilisation photography Textiles Nanomaterials Biosensors Heterogeneous catalyst
Food Industry [52-55]	Dietary ingredient Food preservation (antioxidant, antimicrobial) Emulsify agent
Agriculture [56-62]	Gene elicitor Antibacterial Seed coatings Increases blooms and extends the life of cut flowers

Table 3. Chitosan Chemistry

Purpose	Reaction	Product
Reduce molecular weight (low viscosity, better solubility)	Depolymerisation	Low molecular weight chitosan Chitosan oligomers
Improve cationic properties	Deacetylation Quaternisation Addition of cationic moieties	Chitosan with high DD Quaternised chitosan Highly cationic derivatives
Improve chitosan water solubility	Acylation, alkylation Pegylation Hydroxyalkylation Carboxyalkylation	N-alkyl, acyl-chitosan Peg-Chitosan Hydroxyalkyl-chitosan Carboxyalkyl-chitosan
Amphoteric polyelectrolytes	Carboxyalkylation Phosphorilation Sulfatation	Carboxyalkyl chitosan Phosphonic-chitosan Sulfonic-chitosan
Cell targeting	Alkylation, Crosslinking	Sugar modified chitosan
Photosensible derivatives	Azidation	Azidated-chitosan
Amphiphilic derivatives	Introduction of hydrophobic branches (alkylation, acylation, grafting, crosslinking...)	N-alkyl chitosan Acyl-chitosan Graft derivatives Crown-ether derivatives Cyclodextrin derivatives...
Miscellaneous	Several reactions (thiolation, Michael addition...)	Tiol-chitosan thiourea-chitosan chitosan-dendrimers....

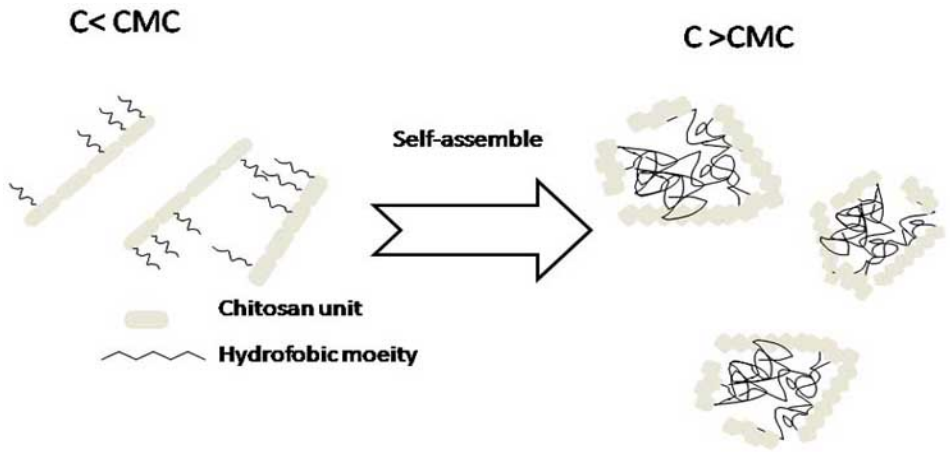

Fig. (2). Self-assembly of amphiphilic chitosan derivatives into micelles.

can also be modified by deacetylation or by the introduction of hydrophobic moieties that alter chitosan hydrogen bonds among other interactions. Since relevant chitosan properties (e.g. mucoadhesive properties, absorption enhancer) depend on the presence of a positive charge on the polymer, chitosan has been modified to introduce non-pH dependent positive charges on the chitosan backbone. To prepare amphiphilic chitosan derivatives, hydrophobic moieties have been introduced into the chitosan backbone by using different methodologies such as alkylation, acylation and graft polymerisation among others.

Amphiphilic chitosan derivatives are soluble in both organic and aqueous solvents and under appropriate conditions they are able to self-assemble. In contact with aqueous solvents, the molecule chains tend to reorganise forming micelles or micelle-like aggregates via undergoing intra- or intermolecular association between hydrophobic moieties, primarily to minimise interfacial free energy. (Fig. (**2**)). Critical micelle concentration (CMC) and Critical Aggregation Concentration (CAC) are two terms that will be frequently used along this paper, therefore we consider that both terms need to be defined. Critical micelle concentration (CMC) is the minimum required concentration for a selected polymer to from micelles through self-assembling. Critical aggregation concentration (CAC) is defined as the minimum required concentration for a selected polymer to aggregate. Normally, CMC values are higher than CAC values. These parameters are very critical indicators of micellisation ability and micelle stability. Micelles are subject to extreme dilution upon intravenous injection into humans. If kinetically stable, slower dissociation allows polymeric micelles to retain their integrity and perhaps drug content while circulating in the blood above or even below CMC/CAC for some time. Thus, a

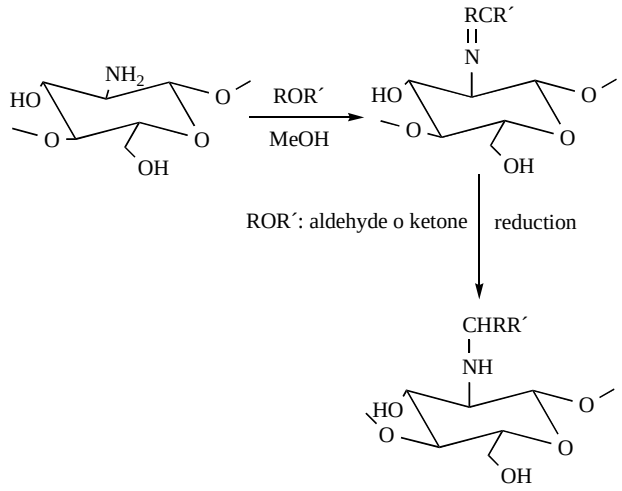

Fig. (3). Synthesis of N-alkyl chitosans.

lower CMC/CAC can warranty that the micelle will retain its original morphology until reaching the target site, which is a significant advantage of amphiphilic polymers over small molecular surfactants.

2.1. N- Alkyl-Chitosans

The primary amino groups of chitosan undergo Schiff reaction with aldehydes and ketones to yield the corresponding aldimines and ketimines, which are converted to an N-alkyl derivative by reduction with sodium borohydride ($NaBH_4$) or sodium cyanoborohydride ($NaBH_3CN$) among other reducing agents (Fig. (**3**)). The choice of the reducing agent is crucial to the success of the reaction, since the reducing agent must reduce imines selectively. $NaBH_3CN$ is widely used in reductive alkylation systems because it is more reactive and selective than standard reducing agents. However, it is highly toxic and generates toxic by-products such as HCN or NaCN. Therefore, the use of this reducing agent is not acceptable in green synthesis [66, 67].

The existence of hydrophobic interactions between the alkyl chains improves the physico-chemical properties of the solutions of modified chitosans. Rheological studies have been carried out with alkyl-chitosans to study these interactions. The influence of the polymer concentration and temperature on the hydrophobic interactions as well as the application of alkyl chitosans as rheological modifiers has been studied by Debrières [66]. Alkyl chitosans with different chain lengths (C3, C6, C8, C10 and C12) and different degrees of substitution were studied. Threshold concentrations, over which the polymer solutions had peculiar temperature behaviour, were defined. According to their structure it was possible to prepare solutions with constant viscosity or with an increasing one with temperature and to develop such derivatives in cosmetics, food or oil industries, for example, as rheological modifiers. However, it was not possible to determine a pertinent parameter that determines the rheological behaviour of alkylated chitosans (length of the grafted alkyl chain, the degree of substitution or both). Rinaudo *et al.* has studied the bulk and interfacial properties of a series of alkylated chitosans having different alkyl chain lengths (C3,C6,C8,C10, and C12) and two degrees of substitution, 2% and 5% [68]. The optimum alkyl chain length was C12 and the degree of grafting 5% to get physical gelation based on the formation of hydrophobic domains. The cross-linking was essentially controlled by the salt concentration. Hydrophobic interactions produced highly non-Newtonian behaviour with large thinning behaviour; this behaviour was suppressed in the presence of cyclodextrins able to cap the hydrophobic alkyl chains. The interfacial properties of the chitosan derivatives were tested for the air/aqueous solution interfaces. Specifically, the role of their structure on the kinetics of film formation was examined, showing that an excess of external salt favours the stabilisation of the interfacial film. The derivatives with a higher degree of substitution and longer alkyl chains were more efficient and gave a higher elastic modulus compared to the model surfactant as a result of the chain properties. Moreover, the aggregation properties in solution of a series of alkyl chitosans with different chain lengths (C5,C6,C8,C10 and C12) have also been studied [69]. In diluted solution, the grafting of a short chain of five carbon atoms is not yet able to hydrophobically promote intra-aggregation of the polymer molecules. In contrast, making the chitosan structure more rigid can improve the polymer–solvent interaction, as shown by its bigger hydrodynamic radius and higher CAC value. By increasing the length of the aliphatic chain, this rigidity effect begins to be partially compensated, for example if a six carbon atoms chain is present. The entropic gain due to intra- and inter-hydrophobic interactions becomes pre-eminent for longer chains. On the other hand, in concentrated solutions, the aliphatic chains and the acetytic units of chitosan promote the formation of local environments whose hydrophobicity is comparable to that of liquid hydrocarbon, thus showing that in concentrated solution the specificity of the grafted chains of different length is lost.

Apart from rheological studies and the application of chitosan alkyl derivatives as rheological modifiers, especially in aqueous-based formulations in various industrial domains such as paints, oil recovery, cosmetics and food, alkyl chitosan derivatives have been used in drug delivery systems, tissue engineering and several technological applications (Tables **4** and **5**).

Chitosan scaffolds for tissue engineering have been fabricated by application of thermally induced phase separation from aqueous solutions of N-butyl-chitosan [70]. The scaffolds produced from N-butyl chitosan were less stable and rigid and possessed average pore diameters that were generally larger than those based on chitosan. Among the scaffolds fabricated from the butyl-modified chitosan, those produced at -20 ºC yielded the most uniform pore structure, the smallest average pore diameters, and the least temporal broadening of pore size distribution.

Klobatzbach *et al.* modified chitosan with butanal, hexanal, octanal, or decanal aldehydes to prepare a biocompatible and biodegradable hydrophobic chitosan membrane that can replace Nafion® for electrode coatings in both sensor and fuel cell applications [71, 72]. Several enzymes such as glucose oxidase, alcohol dehydrogenase, formate dehydrogenase, lactic dehydrogenase, glucose dehydrogenase and formaldehyde dehydrogenase were successfully immobilised and voltammetric studies were carried out. This is the first evidence that hydrophobically modified chitosan can be used at the anode of a biofuel cell.

N-dodecyl-chitosan has been prepared from the corresponding aldehyde using sodium cyanoborohydride as reducing agent [73]. The interaction between this derivative and surfactant vesicles or wormlike micelles was studied as a model for biological membrane interaction. Adding this polymer to surfactant vesicles results in a gel in which the vesicles remain intact. The results suggest the formation of a gel structure in which the vesicles are connected by polymer chains in a three-dimensional network. Vesicle-polymer binding is expected to occur via the insertion of polymer hydrophobes into the vesicle bilayer. Each vesicle thus acts as a multi-

Table 4. Applications of N-Alkyl Derivatives

Application	Reference
Tissue engineering (scaffolds)	[70]
Sensor and fuel cell applications (membranes)	[71,72]
Model study of interaction with biological membranes	[73, 74]
Antibacterial coating	[75]
DNA delivery	[76, 83-84]
Encapsulation of neutraceuticals, cosmetic products	[77,78]
Drug delivery (DOX, PTX, 10H CPT)	[79-81,85]
Membrane coating	[88]

Table 5. Main Properties of Micellar Systems Based On Alkyl-Chitosan Derivatives for Drug Delivery

Chitosan Derivative	DS, %	CAC/CMC mg/ml	Micelle Size, nm	Drug Loading, %	EE, %	Load Molecule
3-O-dodecyl-D-glucose chitosan	9.8-27	$1.2*10^{3}$, 21.8	---	---	---	---
N-succinyl-N-octyl chitosan	28-58 14-39	$0.6\text{-}3*10^{-5}$	100-200	23-36	---	Doxorubicin
N-octyl-O sulfate chitosan	---	0.45	240	25	59	Paclitaxel
N-octyl O,N-carboxymethyl chitosan	55-58 128-138	8-27	200-240	32	80	Paclitaxel
N-Octyl-N-trimethyl chitosan	8-58 54	---	24-280	4.1-32%	7-56	10-hydroxycamptothecin

DS: Degree of substitution. EE: Encapsulation efficiency.

functional junction in the network structure. Significantly, gel formation does not occur with the native chitosan. Moreover, adding the hydrophobically modified chitosan to a viscous sample containing wormlike micelles increases the viscosity further but does not give rise to a gel-like response. Thus, the formation of a robust gel network requires both the presence of hydrophobes on the polymer and vesicles in solution. It has also been proved that the polymer induces transitions in vesicles morphology from unilamellar vesicles to bilamellar ones [74].

Hexanal and aldehyde-terminated PEO chains were simultaneously attached to a low Mw chitosan hydrochloride via reductive amination [75]. The surfactant polymers with PEO/hexyl ratios of 1:3.0 and 1:14.4 were used as surface modifying agents to investigate their anti-infection properties. *E. coli* adhesion on Silastic® surface was decreased significantly by the surfactant polymer with PEO/hexyl 1:3.0. Surface growth of adherent *E. coli* was effectively suppressed by both tested surfactant polymers.

3-O-dodecyl-D-glucose has been linked to chitosan via reductive N-amination in the presence of sodium cyanoborohydride ($NaBH_3CN$) [76]. CAC measurement in acidic media by using two derivatives with different degrees of substitution (9.8 and 27%) suggested that the stability of the polymer micelle was highly dependent on the density of hydrophobic substituents. This derivative was proposed to be used for drug and DNA delivery as well as an extractor solution to dissolve hydrophobic compounds.

Onessipe and Lagerge have studied the interactions between N-alkyl Chitosan (C12) and SDS. Their findings demonstrate that alkylated chitosan/SDS system could be used as wall material for capsules using the coacervation process [77, 78]. Alkyl side chains offer better surface active properties to surfactant polyelectrolyte complexes and formation of complexes for lower SDS concentration than chitosan/SDS system. These capsules may be useful for the design of chitosan-based food ingredients with specific functional characteristics or for the encapsulation of hydrophobic molecules in the cosmetic field.

N-succinyl-N-octyl chitosan (SOCS), which can form micelles in an aqueous media, has been prepared by modifying the amino group with a hydrophobic long chain alkyl functionality and a hydrophilic succinyl moiety [79]. Doxorubicin (DOX), a model antitumour drug, was successfully loaded into SOCS micelles and a sustained release pattern was observed. The *in vitro* anti-tumour activity studies indicated that DOX loaded SOCS micelles were more cytotoxic than free doxorubicin.

N-octyl-O sulfate chitosan (OSCS) has been prepared in a two step process [80]. In the first step, an octyl moiety was introduced via reductive amination using KBH_4 as reducing agent. In the second step, N-octyl chitosan was suspended in N,N dimethylformamide (DMF) and a solution of chlorosulfonic acid in DMF was added. The micelles were used for the sustained released of Paclitaxel (PTX). The formation and loading of the micelles occurred simultaneously in the dialysis process when ethanol and water were used as the solvents for PTX and the polymer, respectively. TEM photograph revealed that PTX existed as colloid particles in ethanol before loading and in the cores of the spherical polymeric micelles

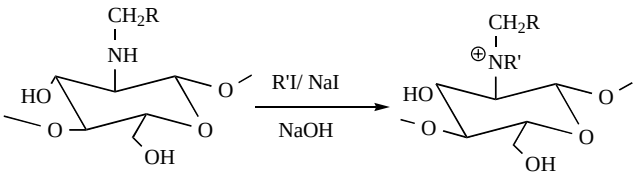

Fig. (4). Quaternisation of chitosans by using alkyl iodides.

after loading. Biodistribution studies indicated that most of the PTX was distributed in liver, kidney, spleen and lung and the longest retention effect was observed in the lung. PTX-M had the similar antitumor efficacy as PTX, but significantly reduced its toxicity and improved the bioavailability [81]. In a further study, the LD_{50} value of OSCS administrated by i.v. and i.p. were calculated as 102.59 and 130.53 mg/kg, respectively. No intravenous stimulation, injection anaphylaxis, hemolysis or cytotoxicity were observed in the safety studies [82].

2.2. Quaternisation

The objective of this modification is to introduce permanent positive charges along the polymer chains, conferring a cationic polyelectrolyte character independently of the aqueous medium pH. Usually, quarterisation is carried out in basic media using alkyl iodides (Fig. (**4**)).

Methylated N-(4-N,N-dimethylaminobenzyl) chitosan has been prepared via reductive amination with 4-N,N-dimethylamino benzaldehyde and $NaCNBH_3$. In a second step, the hydrophobic derivative was methylated by reaction of methyl iodide in alkaline solution [83, 84]. The transfection efficiency of quaternised N-(4-N,N-dimethylaminobenzyl) chitosan, TM-Bz-CS, using the plasmid DNA encoding green fluorescent protein (pEGFP- C2) on human hepatoma cell lines (Huh7 cells) was studied. The rank of transfection efficiency of the chitosan derivatives depends on the degree of methylation as well as the degree of substitution. The pH of the culture medium did not affect the transfection efficiency of the chitosan derivative/DNA complex, whereas it did affect the transfection efficiency of chitosan/DNA complex. Moreover, the polymer was safe at the concentration of the highest transfection. The results indicated that the improved gene transfection was due to the hydrophobic group (N,N-dimethylaminobenzyl) substitution on chitosan which promoted the interaction and condensation with DNA as well as N-quaternisation which increased chitosan water solubility and enhanced gene expression.

Following a similar strategy of quaternisation with alkyl iodides, N-Octyl-N-trimethyl chitosan derivatives (OTMCS) have been prepared [85]. The possible use of OTMCS derivatives in solubilisation and controlled release of 10-hydroxycamptothecin (10-HCPT), a hydrophobic anticancer drug, was studied. The release behaviour of the 10-HCPT-OTMCS micelles was measured and compared to that of a commercial 10-HCPT lyophilized powder *in vitro* and *in vivo*. The solubility of 10-HCPT in aqueous fluid was increased about 80,000-fold from 2 ng/ml in water to 1.9 mg/ml in OTMCS micellar (degree of octyl and trimethyl substitution is 8% and 54%, respectively) solution. In addition, OTMCS was able to modulate the *in vitro* release of 10-HCPT, improving its pharmacokinetic properties and the stability of the lactone ring *in vivo*.

N-dimethyl and N-trimethyl chitosan from octyl, decanal and lauryl chitosan have been prepared [86]. Both, chain length of the N-alkyl group and degree of quaternisation were able to alter the micelle forming properties of the N-alkyl-N-methyl chitosan derivatives. N-octyl-N-dimethyl and N-octyl-N-trimethyl chitosan derivatives formed polymeric nanomicelles, whereas the corresponding N-decanal and N-lauryl chitosan derivatives with the same trimethylation degree formed polymeric micromicelles with 2-5 times greater diameters. CMC was 0.43 mg/ml for the N-octyl-N-dimethyl chitosan derivatives with an N-octyl substitution degree of 8% and a trimethyl substitution degree of 27%.

De Britto and de Assis have recently described a new method to synthesise quaternary salts of N-alkyl chitosan by using dimethyl sulfate [87] (Fig. (**5**)). Dimethyl sulfate is considerably less expensive than iodomethane and is less toxic. In addition, it also has a high boiling point and no solvent is required for the reaction. By using this methodology, N-alkyl chitosan films (C4, C8 and C12 carbon moieties in the polymeric chain) have been prepared by solvent-casting [88]. The average degree of quaternisation of these N-methylated derivatives was calculated to be 35%. Chitosan and its derivatives films are typically brittle materials, exhibiting similar non-linear viscoelastic behaviours. The films of unmodified chitosan have a very small strain, though they were the most resistant ones. In general, the alkyl-chitosan derivatives appear to be more plastic than chitosan films but less resistant. Conversely, the quaternisation reaction increased the hardness of the parent sample.

2.3. Acyl Chitosan

Comparing N-alkylation with acylation, the later is more versatile since it allows the introduction of hydrophobic moieties at amino, alcohol or both residues. Moreover, the introduction of a hydrophobic moiety with an ester linkage allows the action of lipase-like enzymes being these derivatives very interesting as biodegradable materials. N-acyl chitosan derivatives with different purposes have been synthesised as shown in Table **6**.

2.3.1. N-acyl Chitosan

N-acyl chitosan derivatives are obtained from anhydrides (linear or cyclic), acyl chlorides and lactones as well as by coupling carboxylic acids mediated by carbodiimine, usually 1-ethyl-3-(3 dimethylaminopropyl carbodimide (EDC), or EDC and N-hydroxysuccinimide (NHS)). In this reaction, EDC converts the carboxylic acid into a reactive intermediate which is susceptible to

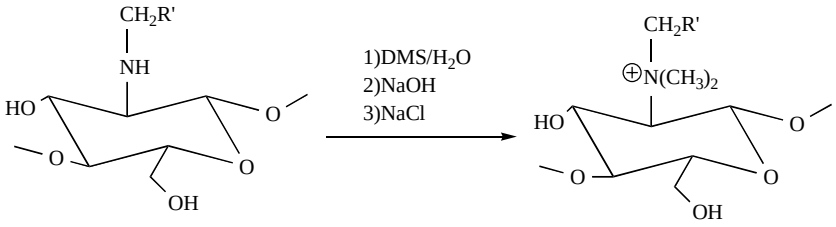

Fig. (5). Quaternisation of chitosans by using dimethylsulfate.

Table 6. Applications of Acyl-Chitosan Derivatives

Application	Reference
Drug delivery (hydrophobic and hydrophilic drugs)	[90, 93,95,99,102-104,107-109,122-123]
DNA delivery	[96,100,106]
Polymeric surfactants, foaming forming agents	[91]
Artificial viscosifiers (biomedical and pharmaceutical application)	[91]
Dispersant /coating of nanoparticles, (biolabeling and biosensoring)	[92, 98]
Antibacterial activity (biomedicine)	[96]
Smart materials	[97, 110-112]
Tissue engineering	[101,120]
One-step purification of IgG	[105]
LB layers	[118,119]

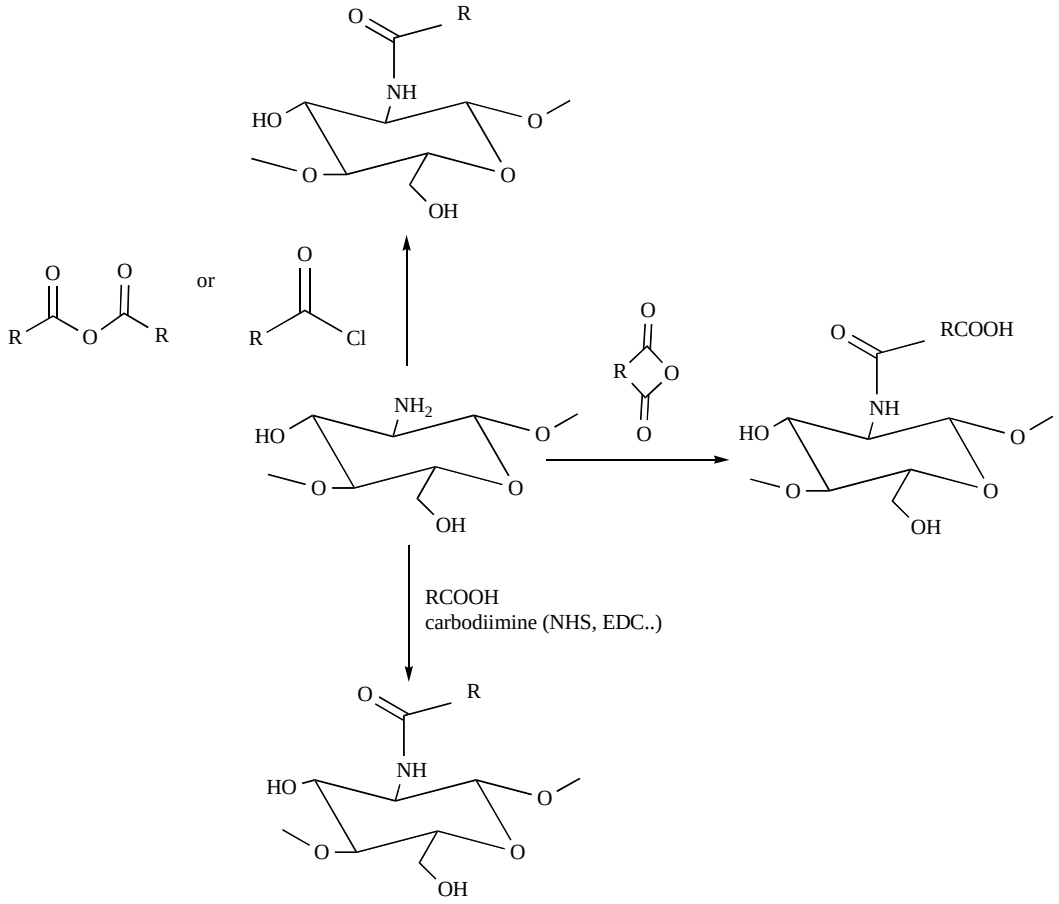

Fig. (6). Synthesis of acyl-chitosans.

attack amines. NHS can help the reaction by producing a more stable reactive intermediate (Fig. (**6**)).

2.3.1.1. Acyl Derivatives from Anhidrides

N-succinyl-chitosan (NSCS), with a well-designed structure and the ability to self-assemble in regular nanosphere morphology, has been successfully synthesised [89]. The *in vitro* cell culture indicates that NSCS is non-toxic and has cell-compatibility. The interactions between NSCS and bovine serum albumin (BSA) were studied [90]. It has been demonstrated that BSA binds to NSCS with a molar ratio of 30:1. This study demonstrates the potential of NSCS matrix for the encapsulation of proteins or other hydrophilic bioactive drugs.

N-[2(3)-(dodec-2´-en-1´-yl) succinoyl] chitosan (DDC-chitosan) of varying degree of substitution (5-30% mol) has been synthesised by the reaction of chitosan with (2-dodecen-1-yl) suc-

cinic anhydride in 1% acetic acid/methanol (1:1 v/v) solution [91]. Surface tension activity, rheological and foam-forming properties of DDC-chitosan were strongly determined by the degree of substitution. Viscosity experiments revealed inter/intramolecular interactions of DDC-chitosan macromolecules in aqueous solutions. These interactions were most pronounced at substitution degrees higher than 10 mol%, while the surface tension activity and the foam-forming ability of DDC-chitosans were maximal at a substitution degree of 5 mol.%. Amphiphilic DDC-chitosans, at low concentrations can be used as promising polymeric surfactants (polysoaps) and foam-forming agents, while concentrated solutions of DDC-chitosans may be useful as artificial viscosifiers for different industrial processes and biomedical and pharmaceutical applications.

N-succinyl-O-carboxymethylchitosan (NSOCMCS) has been proved to be an excellent dispersant by preparing a well-dispersed suspension of superparamagnetic Fe_3O_4 nanoparticles due to its amphiphilic polyelectrolyte property [92]. NSOCMCS can be easily chemisorbed onto Fe_3O_4 nanoparticles by the formation of chelation complexation. The adsorbed NSOCMCS stabilises the Fe_3O_4 nanoparticles, a process which is driven by both steric and electrostatic stabilisation mechanisms. Moreover, the NSOCMCS/ Fe_3O_4 nanoparticles have been proved to have good cytocompatibility, which is important for further biomedical applications. NSOCMCS, with functional carboxyl groups, can be incorporated in drugs to provide time-specific and site specific drug delivery through the modification of the NSOCMCS shell on Fe_3O_4 nanoparticles with stimuli-responsive functional groups or specific tissue-targeting ligands.

Carboxymethyl-hexanoyl Chitosan (CMHCS) hollow nanocapsules have been developed in aqueous system without the aid of surfactants, organic solvents, emulsion phases, or template cores [93]. The results of SEM and TEM proved that the self-assembled CMHCS nanocapsules exhibited spherical morphology and a core-shell configuration. The formation of CMHCS self-assembled hollow nanocapsules is due to the hydrophobic interactions of hexanoyl groups in aqueous solution. The negatively charged carboxymethyl groups also play an important role in the morphology and the stability of nanocapsules. The loading of doxorubicin can be improved by an increase of hexanoyl substitution and a sustained release over a reasonable period of time can be achieved.

Stearoyl, palmitoyl and octanoyl chitosan derivatives with degrees of substitution from 0.9% to 29.6% have been prepared [94]. The N-fatty acylations were carried out by reacting carboxylic anhydride with chitosan in dimethyl sulfoxide. The chitosan derivative-based micelles were spherical in shape being their sizes in the range of 140–278 nm. The CAC can reach $1.99*10^{-3}$ mg/ml, The properties of palmitoyl-chitosan micelles such as encapsulation capacity and controlled release ability of hydrophobic model drug ibuprofen were evaluated [95]. Experimental results indicated that the loading capacity of Palmitoyl-CS was approximately 10%. The drug release strongly depended on pH and temperature; low pH and high temperature accelerated drug release markedly.

Chitosan has been selectively N-acylated with acetic, propionic and hexanoic anhydrides under homogeneous conditions to prepare N-acetyl chitosan (NACS), N-propionyl chitosan (NPCS) and N-hexanoyl chitosan (NHCS), respectively [96]. NACSs with different N-acetylation degrees were obtained by controlling the degree of N-acetylation. Intramolecular aggregation of NPCS and NACS was stronger with NPCS than NACS. Hydrophobic interaction of N-acylated chitosan substituted with longer acyl chains was stronger. With moderate DD, intramolecular aggregation occurs predominantly. In vitro antibacterial activity of N-acylated chitosans was evaluated against two Gram-positive bacteria and two Gram-negative bacteria. Relative inhibition time of NHCS with a concentration of 1 mg/ml against *Escherichia coli* and *Pseudomonas aeruginosa* was more than 2-6 times longer than that of NACS and NPCS. N-acylated chitosan with lower DD had a higher inhibitory effect on the growth of bacteria than that with moderate DD.

Gel formation during the N-isobutyrylation of chitosan has been investigated as a function of the reaction stoichiometry (R), chitosan concentration, and temperature by small deformation oscillatory rheology [97]. An excess of isobutyric anhydride was suggested to be necessary for nucleation and hydrophobic association. The potential application of N-isobutyrylchitosan (NIBCS) hydrogels in the design of thermally sensitive materials was also demonstrated.

Oleoyl chitosan and a thiol-chitosan derivative have been used for coating gold nanospheres and nanorods [98]. The multiple thiol and oleyl groups in the polymer backbone provided multiple binding sites to the nanoparticles for a robust coating protection. The polymer-coated nanoparticles could be further functionalised with biomolecules for applications such as biolabeling and biosensing.

2.3.1.2. Acyl Chitosan Derivatives from Acyl Chlorides

Oleoylchitosans (OCS) have been synthesised by reacting chitosan with oleoyl chloride [99]. The hemolysis rates of OCS nanoparticles tested in different conditions came well within permissible limits (5%). The OCS nanoparticles showed no cytotoxicity to mouse embryo fibroblasts. Doxorubicin (DOX) has been efficiently loaded into OCS nanoparticles (encapsulation efficiency: 52.6%). The drug was rapidly and completely released from the nanoparticles (DOX-OCS nanoparticles) at pH 3.8, whereas at pH 7.4 there was a sustained release after a burst release. The inhibitory rates of DOX-OCH nanoparticle suspension to different human cancer cells (A549, Bel-7402, HeLa, and SGC-7901) significantly outperformed that of DOX solution.

2.3.1.3. Acyl Chitosan Derivatives from Coupling Reactions

Deoxicolic acid (DOCA) has been used to modify chitosan oligosacharides (COS) [100]. To facilitate the coupling reaction with primary amine groups in COS, the carboxylic group in DOCA was activated by converting the carboxylic acid group into NHS-ester form (succinimido deoxycholate, DOCA– NHS). As efficient gene carriers, the COSD nanoparticles showed superior gene condensation and protection of condensed gene from endonuclease attack than unmodified COSs. Furthermore, COSDs showed great potential as gene carriers with high level of gene transfection efficiencies, even in the presence of serum.

An aniline pentamer chitosan derivative (APCS) with electroactivity has been prepared by a coupling reaction [101]. Due to its amphiphilic property, this derivative is able to self-assemble into spherical micelles, which makes the potential application of these polymers in drug delivery possible. The use of these polymers as scaffold materials in neuronal tissue engineering was evaluated; non-cytotoxicity, degradability in the presence of enzymes and biocompatibility were observed. Moreover, differentiation of PC-12 cells seeded on the pure CS and on the three electroactive samples with 2.5, 4.9, and 9.5% AP, respectively, in the presence of exogenous NGF was assessed for up to 5 days. The PC-12 cells on the samples containing AP showed neurite extension and some of them even formed intricate networks while those on the CS showed much fewer neurites.

Quaternary ammonium palmitoyl glycol chitosan self-assembled in aqueous media at low micromolar concentrations to give previously unknown micellar clusters of 100-300 nm in size [102]. Micellar clusters comprise smaller 10-30 nm aggregates, and the nanopolarity/drug incorporation efficiency of their hydrophobic domains can be tailored by varying the degree of lipidic substitution and molecular weight of the chitosan. The extent of drug incorporation by these novel micellar clusters is one order of magnitude higher than what is observed with triblock copolymers, with molar polymer/drug ratios of 1:48 to 1:67. On intravenous injection, the pharmacodynamic activity of a carbohydrate propofol formulation is increased by one order of magnitude when compared to a commercial emulsion formulation. On topical ocular application of a carbohydrate prednisolone formulation, initial drug aqueous humour levels are similar to those found with a 10-fold dose of prednisolone suspension.

Linoleic acid has been covalently conjugated to chitosan or chitosan derivatives *via* a 1-ethyl-3-(3-dimethylaminopropyyl)-carbodiimide-mediated (EDC-mediated) reaction to generate amphiphilic chitosan derivatives [103-105]. Linoleic-chitosan nanoparticles have been used for the adsorption of trypsine (TR) [103]. Environment factors (e.g. pH, concentration of urea or NaCl) can affect TR loading on the nanoparticle. The thermal stability of TR loading on nanoparticles was significantly improved compared to free TR. On the other hand, adriamycin (ADR) has been physically entrapped in self-aggregates based on linolenic-carboxyl-chitosan derivatives [104] The drug loading experiments indicate that the loading capacity and efficiency increase with increasing concentration of ADR. ADR is slowly released from chitosan self-aggregates for about 3 days. Furthermore, the effects of drug controlled release become more obvious on increasing the pH value. Linoleic acid attached chitosan beads [poly(LA-Ch)] have been obtained by the formation of amide linkages between linoleic acid and chitosan [105]. Poly(LA-Ch) beads were used for the purification of immunoglobulin-G (IgG) from human plasma in a batch system.

Stearic acid has also been coupled to chitosan via activation with EDC for DNA and drug delivery applications [106-112]. Stearic acid-chitosan oligosaccharides micelles showed a CMC of 0.035 mg/ ml (DS 25.4%) [106]. Due to their cationic properties, the micelles could compact the plasmid DNA to form micelle/DNA complex nanoparticles, which can efficiently protect the condensed DNA from enzymatic degradation by DNase I. The IC_{50} value of the CSO–SA micelle against A549 cells was 543.16 μ/ml, while the IC_{50} of LipofectamineTM 2000 was about 6 μg/ml. The *in vitro* transfection efficiency of CSO–SA micelles was investigated by using plasmid DNA (pEGFP-C1). The transfection of CSO–SA was not interfered in the presence of 10% fetal bovine serum, which showed a remarkable enhancement effect. The optimal transfection efficiency of CSO–SA micelles in A549 cells was about 15%, which was higher than that of CSO (about 2%) and nearer to that of LipofectamineTM 2000 (about 20%).

Stearic acid grafted chitosan oligosaccharide (CSO-SA) with different degree of amino substitution (5, 12 and 42%) has been synthesised with a CMC of about 0.06, 0.04, 0.01 mg/ml, respectively [107]. Paclitaxel was incorporate into the micelles, and the surface of the micelles was further cross-linked by glutaraldehyde to form drug loaded and shell cross-linked nanoparticles. The drug release rate decreased with the increase of SD of CSO-SA and cross-linking degree. Doxorubicin has also been loaded into stearic CSO-SA micelles with 3.48% amino-substituted degree (CMC= 0.035 mg/ml) [108]. The CSO-SA self-aggregate micelles showed spatial structure with multi-hydrophobic core. A fast cellular internalisation of CSO-SA micelles was observed when using A549, LLC, and SKOV3 cells as model tumour cell lines in cellular uptake studies. To reduce the initial burst drug release from CSO-SA micelles loading DOX (CSO-SA/DOX), the shell of CSO-SA micelles was crosslinked by glutaraldehyde. The cellular inhibition experiments demonstrated that the cytotoxicity of DOX was increased by the encapsulation of CSO-SA micelles. CSO-SA/DOX displayed the best antitumor efficacy in SKOV3 cell line due to the higher cellular uptake percentage of CSO-SA micelles and the lower sensitivity of free drug to the cells. The cytotoxicities of shell crosslinked CSO-SA/DOX were more enhanced in all cell lines than those of unmodified CSO-SA/DOX. Apart from crosslinking of the micelles with glutaraldehyde to reduce the burst effect, the modification of the core of CSO-SA micelles by physical solubilisation of stearic acid (SA) has also been proposed [109]. The *in vitro* drug release tests showed that the incorporation of SA into CSO-SA micelles could reduce the drug release from the micelles due to the enhanced hydrophobic interaction among SA, hydrophobic drug and hydrophobic segments of CSO-SA.

You *et al* has demonstrated that stearic-chitosan micelles show pH-sensitive properties, thus favouring intracellular delivery of encapsulated drug [110-112]. The micelles, with high DS (34.7%), present specific spatial structure with multiple hydrophobic minor cores and thus obtain excellent internalisation into cancer cells and accumulation in cytoplasm as proved by using paclitaxel and doxorubicin as model drugs.

2.3.2. O-acyl Chitosan

Since the amino group is more active than the two hydroxyl groups, a protection is often necessary in order to prepare N,O-acyl chitosan with a refined substitution pattern. Nishimura *et al.* reported the preparation of amphiphilic chitosans by using a N-phthaloyl chitosan as an intermediate [113]. However, this method needs several steps for the protection and deprotection of the amine groups. Seo *et al*. also reported a heterogeneous method to prepare N,O-acyl chitosan by using N-acyl chitosan as a precursor [114]. However, the resultant N,O-acyl chitosans had lower DS and showed poor solubility in organic solvents. At the same time, a new method for selective O-acylation of chitosan in methanesulfonic acid (MSA) was proposed [115]. This method is based on the idea that the amine groups are protonated through formation of a salt with MSA, which is disadvantageous for a nucleophilic displacement reaction. As a result, the substitution occurs preferentially on the hydroxyl groups of chitosan. O-Acyl chitosans are prepared from acyl chlorides in the presence of methanesulfonic acid ($MeSO_3H$) (Fig. (**7**)).

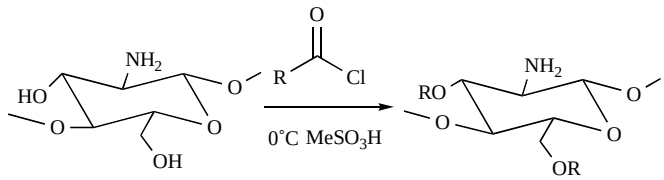

Fig. (7). Synthesis of O-acyl-chitosans.

Acryloyl chitosan (AcCS), has been synthesised by a homogeneous reaction of chitosan and acryloyl chloride using methanesul-

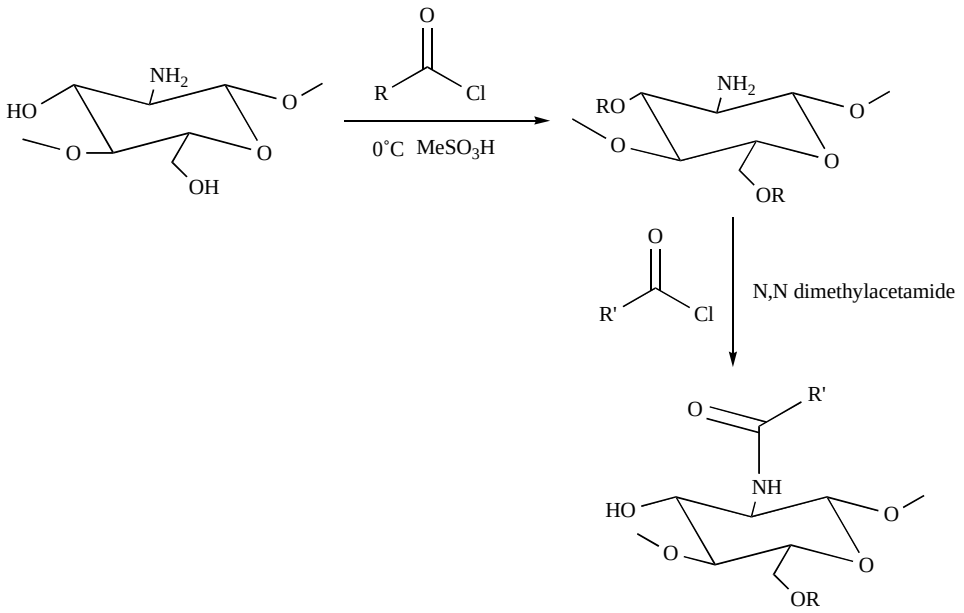

Fig. (8). Controlled synthesis of N,O acyl-chitosans in presence of methanesulfonic acid.

fonic acid as solvent and catalyst [116, 117]. The concentrated solutions of AcCS/acrylic acid were investigated by polarised optical microscopy (POM) and cholesteric mesophase was found above the critical concentration of 45 wt%. AcCS/polyacrylic acid (PAA) composite films were prepared *via* the photopolymerisation of 52 wt% AcCS/acrylic acid solution, and subsequently the permanganic etching technique was employed to expose the textures and defects inside the composite films. The results showed that AcCS/PAA composite films had better anticorrosion capacity than (CS)/PAA composite films, due to the crosslinking network between AcCS and PAA. Therefore, the studied system takes the advantage of easy controlling for permanganic etching.

The regioselective synthesis of an amphiphilic chitosan derivative, O,O,O-dipalmitoyl chitosan (DPCT) has also been achieved in the presence of $MeSO_3H$ [118]. The amphiphilic behaviour and miscibility of DPCT with cholesterol were studied through the Langmuir–Blodgett (LB) technique. The condensed monolayer formed in the water–air interface had a collapse pressure of 40 mN/m and could be transferred onto various substrates by the Langmuir–Blodgett technique. Furthermore, this amphiphilic chitosan derivative with free amino groups was cross-linked with glutaraldehyde. The results showed that this cross-linked amphiphilic chitosan derivative could effectively adsorb cholesterol both in polar and in non-polar solvents.

2.3.3. Controlled N,O-acylation of Chitosan

The O-acyl chitosans synthesised via an MSA protection method generally dissolve in N,N-dimethylacetamide (DMAc). Therefore, further N-acylations could be conducted on the O-acyl chitosans in homogeneous systems using DMAc as solvent (Fig. (**8**)).

O-octanoyl, N-cinnamate chitosan (OCCS) and N-O-hexanoyl chitosan (HCS) derivatives have been synthesised using the aforementioned methodology [119, 120].

A diluted OCCS/chloroform solution could be spread onto the water surface to form a monomolecular layer using LB method. A limiting area of about 100 A^2 was estimated from the p–A isotherm. YZ-type LB films with 10, 20, and 30 layers were deposited onto hydrophobic quartz substrates by a vertical dipping method. The intrinsic chirality originating from the helical backbones of OCCS is maintained in the LB film. The helical conformations of OCCS do not suffer any obvious change during a casting process or a forced organising process by LB method. The main chains of OCCS in the two dimensional LB films are also preferentially orientated.

On the other hand, the effect of HCS on dermal fibroblast attachment, proliferation and viability has been examined *in vitro*, and compared with poly(lactide-co-glycolide) (PLGA) and chitosan. The *in vivo* tissue response to HCS was compared with PLGA implanted subcutaneously. It was found that HCS had no deleterious effect on the viability of dermal fibroblasts. *In vivo*, HCS displayed a favourable tissue response profile compared with PLGA, with significantly less inflammation and fibrosis. The erosion rate of HCS could be modulated by changing the degree of substitution of hexanoyl groups.

N, O selective acylation of chitosan has also been carried out by reacting water soluble chitosan with D, L PLA in DMSO in the presence of triethylamine (Fig. (**9**)) [121]. The CMC value of DLLA/CS (11:1) (DL-lactide/aminoglucoside units molar ratio) in water was 0.065 mg/ml. Recently, Rifampin has been incorporated into these polymeric micelles as a lipophilic model drug to investigate the drug release behaviour [122]. As PLA weight ratio increased, the micelle size and drug-loading content increased while the drug release rate decreased.

N,O amphilphilic derivatives containing N-phthaloyl moieties can be easily prepared by a coupling reaction in the presence of dicyclohexylcarbodiimide [123]. For instance, N-phthaloylchitosan-g-polyvinylpyrrolidone (PHCS-g-PVP) has been synthesised by grafting polyvinylpyrrolidone (PVP) onto a chitosan derivative whose amino groups have previously reacted with phthalic anhydride. Polymeric micelles were prepared by the dialysis method and showed a CMC of 0.83 mg/L. Prednisone acetate was incorporated in the polymeric micelles with a loading capacity of around 45%. *In vitro* tests showed that the release of prednisone acetate from the micelles was continuous with no initial burst.

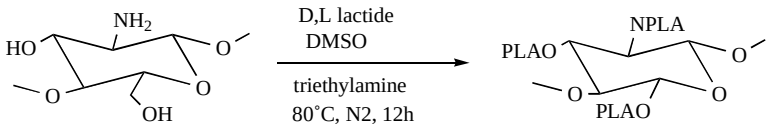

Fig. (9). Controlled synthesis of N,O acyl-chitosans in presence of triethylamine.

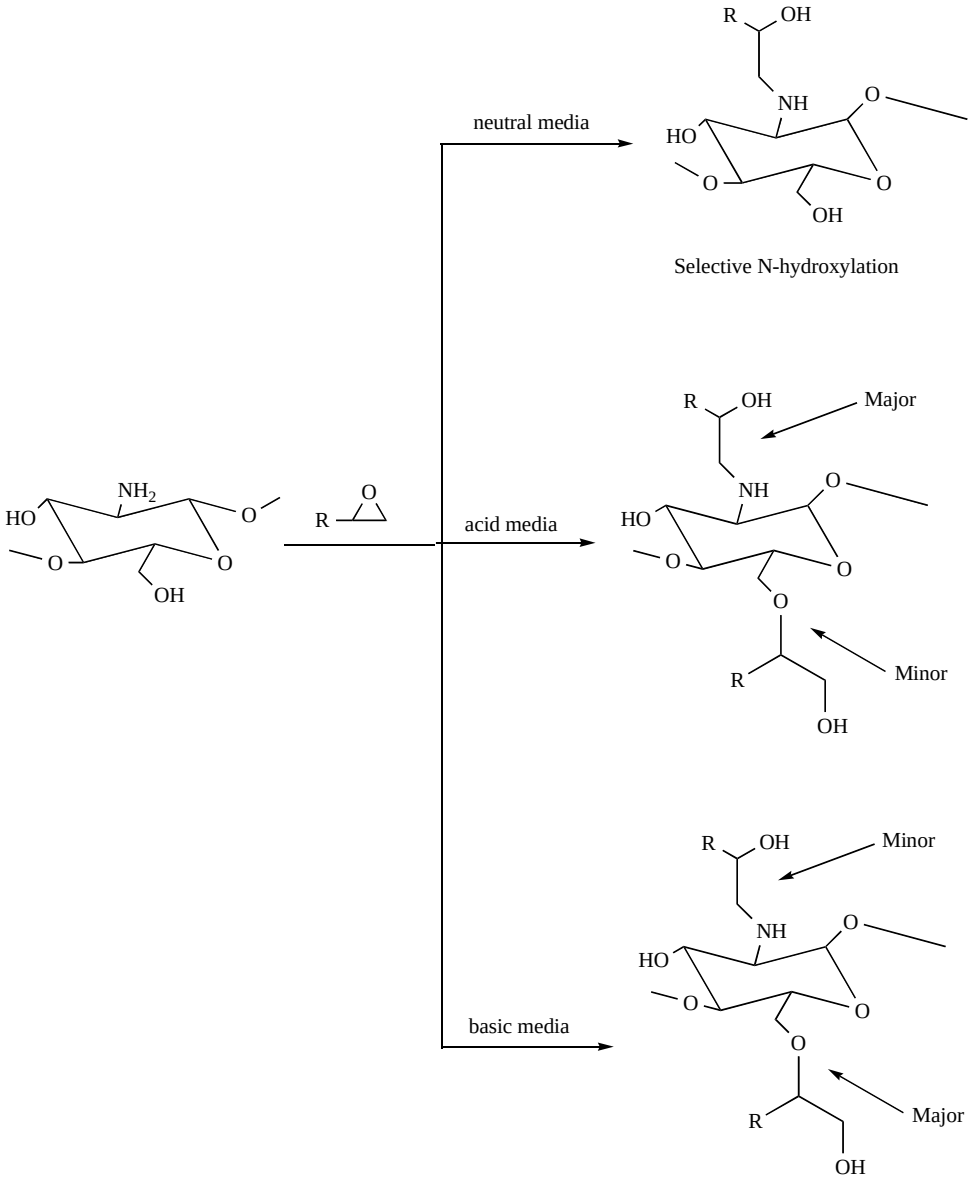

Fig. (10). Synthesis of hydroxyalkyl chitosans.

2.4. Hydroxyalkyl Chitosan

Hydroxyalkyl chitosan derivatives are prepared by reacting chitosan with epoxides. Depending on the reaction conditions (temperature and pH) and on the epoxide used the reaction takes place on the primary amino groups, on the hydroxyl groups or on both (Fig. (**10**)).

Polydimethylsiloxane-modified chitosan has been synthesised by reaction between amine groups of chitosan and epoxy group of polydimethylsiloxane (PDMS) [124]. By using a difunctional glycidoxypropyl terminated PDMS, a crosslinked polymer was prepared. Organic–inorganic hybrid membranes, obtained by simultaneous grafting and crosslinking of chitosan with epoxy-terminated polydimethylsiloxane and γ-glycidoxypropyltrimethoxysilane have also been prepared [125]. Porous membranes have been obtained by acid decomposition of calcium carbonate porogenic agent trapped inside the material. The hybrid membranes were characterised by an expected reduction of water sorption ability due to both the crosslinked structure and the increased hydrophobicity induced by the siloxane grafts. As expected from the low surface tension of siloxane polymers, the modification of chitosan with polysiloxane enhances the surface hydrophobicity of the materials, as indicated by the increase of water contact angles. This derivative has also the ability to complex divalent metal cations [126].

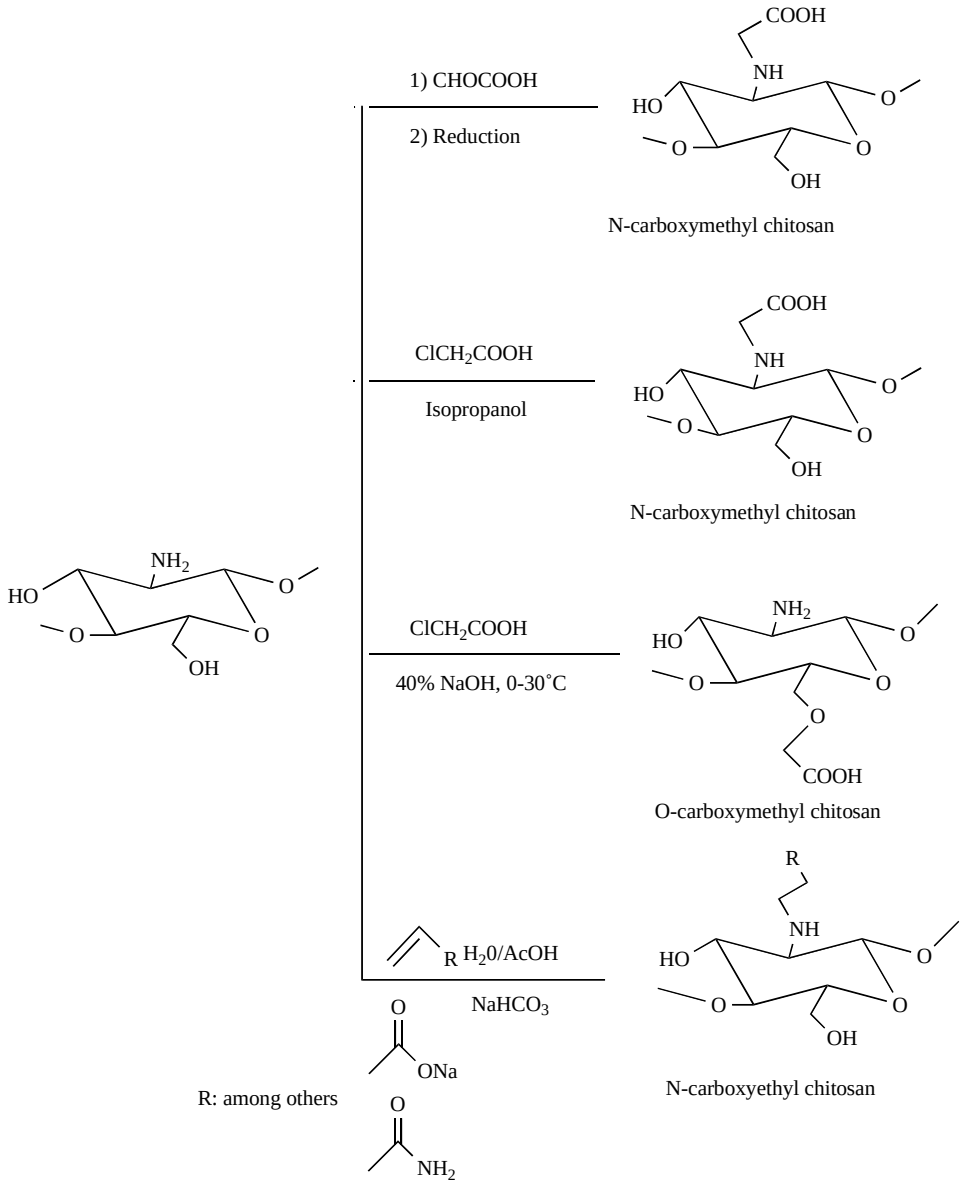

Fig. (11). Synthesis of carboxyalkyl chitosans.

2-hydroxyl-3-butoxyl-propylcarboxymethyl-chitosan (HBP-CMCHS) has been synthesised from a commercial carboxymethyl chitosan [127]. This derivative has been used to study the controlled release of puerarin. The micelles were spherical and puerarin was solubilised in the cores of the spherical polymeric. The CMC of HBP-CMCHS was 0.5 mg/ml when DS of the hydrophobic group was 24%.

Glycol-chitosan is an amphiphilic chitosan derivative, prepared from glycidol, which is soluble in water and able to self-assemble in aqueous media. Apart from its use as amphiphilic derivative, it has been used as starting molecule to synthesise other chitosan derivatives taking advantage of its solubility in water. Hydrophobically modified glycol chitosans capable of forming nano-sized self-aggregates have been prepared by chemical conjugation of fluorescein isothiocyanate (FTIC) or doxorubicin (DOX) to the backbone of glycol chitosan [128]. *In vivo* biodistribution of the self-aggregates was assessed, following systemic administration via the tail vein of the tumour-bearing mice (glycol-FTC-chitosan derivative). The results demonstrated that the distributed amount of self-aggregates gradually increased in the tumour as blood circulation time increased. The self-aggregates loaded with doxorubicin (glycol DOX-chitosan derivative) effectively suppressed the tumour growth *in vivo*. Other molecules that have been linked to glycol chitosan are cholanic acid [129-136], FTIC [137, 138] , palmytoil, hexadecyl [139], N-acetyl cystidine [140] and bile acid [141]. Table **7** summarises the applications of these derivatives.

2.5. Carboxyalkyl Chitosan

N- and O-Carboxyalkylation takes place when chitosan reacts with monohalocarboxylic acids using different reaction conditions to control the selectivity of the reaction (Fig. (**11**)). Carboxyaldehydes have been used to selectively produce N-carboxyalkyl chitosan derivatives by reductive amination. Vinilic polymers (as acrylic acid) have also been used to produce N-carboxyalkyl chitosan derivatives.

The structure of O-carboxymethylchitosan (OCMCS) and its aggregation behaviour in diluted solution have been studied [142].

Table 7. Applications of Glycol Based Chitosan Derivatives

Hydrophobic Moiety	Application	Ref.
Cholanic acid	Cell targeting, drug delivery (Paclitaxel, peptides, docetaxel, camptothecin, cisplatin) Gene delivery	[128-136]
FTIC	Biodistribution studies in drug delivery, morphological studies.	[137, 138]
Palmytoil, hexadecyl	Gene delivery	[139]
N-acetyl-cystidine	Intra-cytoplasmatic drug delivery	[140]
Bile acid	Drug delivery	[141]

The CAC was determined to be between 0.042 mg/ml and 0.050 mg/ml. Camptothecin (CPT) has been loaded in O-carboxymethyl-chitosan (OCMCS) aggregates and the controlled release of the drug has been studied [143]. The results have demonstrated that not only the aggregates but also the unimers of OCMCS can help to enhance the solubility of CPT. After CPT is loaded in OCMCS, its release is significantly sustained, which is caused by the interactions between OCMCS and lipophilic CPT. *In vitro* cancer antiproliferative activity test further confirms the slow release of CPT from OCMCS-drug system. This release system can solve the commonly existing problem of unavailability of assembled micelles from amphiphilic copolymer in the significant dilution accompanying i.v. injection. Gatifloxacin (GFLX) has also been encapsulated into OCMCS nanoparticles [144]. The MIC of OCMCS formulation against Gram-negative bacteria is fourfold lower than that of GFLX solution. However, the MIC of OCMCS formulation against Gram-positive bacteria is similar to that of GFLX solution. OCMCS matrix has obvious "transmission effect" on Gram-negative bacteria. The finding can broaden the applications of GFLX to Gram-negative bacteria.

N-Phthaloyl-carboxymethylchitosan (CMPhCS) has been successfully prepared by reacting N-phthaloylchitosan with chloroacetic acid in isopropyl alcohol [145]. CMPhCS existed as a flexible chain in the aqueous solution and aggregated gradually to form sphere aggregates in the mixture solution of H_2O-DMF. CMPhCS could be self-assembled to form various morphologies of crew-cut aggregates including vesicles, vesicle-encapsulating vesicles, onion-like vesicles, and large compound micelles in the mixture system. For the first time, onion-like self-assembled vesicles from a natural polymer derivative have been observed with TEM. Micelles were self-assembled from N-phthaloyl-carboxymethylchitosan (CMPhCS) in a DMF-H_2O mixture solution, and they were used to evaluate drug deliveries of levofloxacine hydrochloride (Lfloxin). The results indicated that the CMC of CMPhCS in aqueous solution was 0.20 mg/mL. Moreover, the Lfloxin and BSA could be controlled to be release within 72 h in sodium phosphate buffer (pH 7.4) [146].

2.6. Grafted Derivatives

Graft polymerisation is one of the most important methods used to modify chitosan to produce amphiphilic chitosan derivatives.

2.6.1. Graft Copolymerisation by Radical Generation

Graft copolymerisation of vinyl and acryl monomers onto chitosan has been carried out using free radical initiators such as ammonium persulfate (APS), potassium persulfate, ceric amonium nitrate (CAN) and ferrous ammonium sulfate, among others. The polymerisation can also be initiated by gamma radiation or by using enzymes such as tyrosinase. The process consists of the generation of free radicals on the polymer backbone and these radicals serve as macroinitiators for the vinyl or acryl monomer. Graft polymerisation has been used to prepare "smart chitosan derivatives" sensitive to temperature and/or pH to be used in different fields such as biomedicine. Some examples are described below.

A thermosensible polymer has been synthesised by grafting Poly (N-isopropylacrylamide) to water soluble chitosan using CAN as initiator [147, 148]. The ability of human mesenchymal stem cells to differentiate to chondrocytes and cartilage mass formation using the thermosensible material (LCTS 32ºC) was studied and the cartilage formation *in vivo* after injecting a cell-thermosensitive gel complex was evaluated [147]. Although further animal studies are needed, the gel is proposed to be used as an easy method for treating vesicouretral reflux *via* an endoscopic single injection technique (without a dual injection system).

A novel copolymer P(CS–Ma–DMAEMA) has been synthesised with chitosan (CS), maleic anhydride (Ma) and 2-(dimethylamino)ethyl methacrylate (DMAEMA) by grafting and copolymerisation. In a first step, maleic anhydride was grafted to chitosan using dicyclohexyl carboimide (DCC) as a condenser. In a second step, copolymerisation of Ma-Cs and DMAEMA was carried out using $(NH_4)_2S_2O_8$ as the initiator. The aforementioned polymer showed sensitivity to both pH and temperature. Controlled coenzyme A delivery was studied. When pH of the copolymer solution was 7, CoA was largely absorbed by the copolymer. On the other hand, when pH was 3.7, CoA was mostly released from the copolymer. When the temperature of the copolymer solution increased from 35 to 55 ºC, CoA was largely released from the copolymer [149].

Novel cationic hydrogels based on chitosan and 2- hydroxyethylacrylate (HEA) have been obtained by free radical polymerisation of HEA in chitosan water solutions in the presence of the initiator APS and the cross-agent PEGDMA [150]. An interpenetrating structure is proposed for the network with partial grafting of poly-HEA onto the chitosan chains. The interaction between the chitosan–polyHEA hydrogel and the oppositely charged surfactant, sodium dodecyl sulfate (SDS) occurs through electrostatic attraction of the charged amino group and the surfactant head. At higher SDS concentration, namely above the CMC, the formation of a secondary surfactant layer may take place around the primary one through tail-to-tail hydrophobic interaction, or the complex formation may occur directly between the amino groups and the SDS micelles. The process is accompanied by a reswelling of the hydrogels presumably due to their recharging and converting into the anionic network. The modification of the chitosan–polyHEA hy-

drogels with SDS resulted in hydrophobic–hydrophilic balance changes and thereby emergence of pronounced thermo-sensitive properties, while the original networks were practically independent of temperature.

A novel smart drug delivery system (NP-Film) consisting of carboxylated chitosan-grafted nanoparticles (CCGNs) and bilaminated films, which were composed of the mucoadhesive chitosan-ethylenediaminetetraacetic acid hydrogel layer and the hydrophobic ethylcellulose layer, has been developed for oral delivery of protein drugs [151]. Carboxylated chitosan-grafted nanoparticles were prepared by polymerisation of methyl methacrylate onto chitosan backbone using APS as initiator. NP-Film was characterised by electron microscopy and fluorescence microscopy, and the results showed that the solid, spherical nanoparticles were evenly dispersed in the porous structures of the films. The nanoparticles could reversibly open the tight junctions of the intestine and inhibit trypsin activity. The release behaviour of the nanoparticles from the NP-Film exhibited pH sensitivity. The drug delivery system possessed high mucoadhesive force and low intestinal toxicity. In a similar way, a smart polymeric composite carrier consisting of carboxylated chitosan grafted nanoparticles (CCGN) and bilaminated films with one alginate-Ca^{2+} mucoadhesive layer and one hydrophobic backing layer (ethylcellulose) has been prepared [152]. The polymeric carrier possessed high mucoadhesion, good encapsulation capacity for hydrophilic peptides such as calcein, and pH-sensitivity; release of the nanoparticles and calcein from the carrier was restricted in acidic environment while a fast and complete release was achieved in neutral medium. Therefore, with the combined benefits of a mucoadhesive drug delivery system and a nanoparticulate delivery system, the CCGN-loaded bilaminated hydrogel films might have promising applications for oral delivery of peptide drugs.

Magnetic core-shell (MCS) particles consisting of hydrophobic poly(methyl methacrylate) cores with hydrophilic chitosan shells and γ-Fe_2O_3 nanoparticles inside the cores were prepared via copolymerisation of methyl methacrylate from chitosan in the presence of vinyl-coated γ-Fe_2O_3 nanoparticles [153]. The MCS particles were less than 200 nm in diameter with a narrow size distribution and had a good colloidal stability. Magnetisation study of the particles indicated that they exhibited superparamagnetism at room temperature. The MCS particles were able to form a continuous film on a glass substrate, where magnetic nanoparticles could evenly disperse throughout the film. This is an environmentally benign method since the MCS particles are synthesised in the absence of any surfactants, emulsifiers, or toxic organic solvents. It is also a versatile approach because the MCS particles with different core and shell materials can be easily prepared.

Organosilica-chitosan nanospheres have been synthesised in a one-pot way, based on synergic grafting polymerisation, sol–gel reaction and amphiphilic self-assembly. The 'organosilica' core was formed by hydrolysis and condensation of TMSPM that was simultaneously polymerised using -NH_2/TBHP as redox-pair initiator. The nanospheres had adjustable sizes below 100 nm, depending on the ratio of TMSPM to chitosan. This method simplified the preparation of silica-polymer nanospheres by eliminating the previous core-forming step, and by employing natural biopolymer as shell materials. Since a large quantity of bio-molecules contain –NH_2 groups (e.g. gelatin and casein), this method sheds new light on the preparation of a variety of silica-biomolecule hybrids. Thus, synthesised organosilica-biopolymer nanospheres do not have harmful residues, and could be applied in heterogeneous catalysis, gene delivery and antibacterial technologies [154].

Chitosan-g-poly(vinyl acetate) copolymers have been synthesised by the CAN free radical technique. The hydrolysis of the chitosan-g-poly(vinyl acetate) with aqueous sodium hydroxide solution led to chitosan-g poly(vinyl alcohol). Both the copolymers showed enhanced mechanical properties with tunable hydrophilicity. The hydrolysed copolymer was more hydrophilic than its precursor. The increased mechanical properties of the chitosan-g-poly(vinyl acetate) is highly beneficial for the fabrication of strong, degradable matrices. Chitosan-g-poly(vinyl acetate) films are promising candidates for varied engineering and medical applications. The synthetic– natural hybrid copolymers with good mechanical properties and properly tailored hydrophilic/ hydrophobic characteristics could serve as promising candidates for potential applications demanding a strong but degradable and pH sensitive membrane [155].

Gamma-ray irradiation has been used as an initiator to graft synthetic monomers, viz. hydroxyethyl acrylate, hydroxyethyl methacrylate, and N-vinylpyrrolidone to chitosan [156]. The interaction of grafted chitosan polyelectrolyte with SDS was studied as a model of polymer-surfactant interactions in biological tissues. It was found that turbidity of aqueous solutions of grafted chitosan with added SDS exhibits a sudden rise and a subsequent sharp fall due to the aggregation, deaggregation and precipitation of complexes depending on the SDS/cation molar ratio. Chitosan–polyvinyl pyrrolidone hydrogels with pH sensitivity have also been prepared by using ^{60}Co gamma-irradiation [157].

2.6.2. Opening Ring Polymerisation

Chitosan/polycaprolactone graft copolymers of various compositions have been synthesised via a protection graft-deprotection procedure by the ring-opening graft copolymerisation of caprolactone onto PhCS using tin(II) 2-ethylhexanoate as a catalyst (Fig. (**12**)) [158]. Toluene seems to play an important role as a swelling agent to favour the grafting reaction in this heterogeneous system. After deprotection, the phthaloyl group was removed and the amino group was regenerated. Thus, the obtained chitosan-g-polycaprolactone was an amphoteric hybrid with unique free amino groups and hydrophobic polycaprolactone side chains. The polymer exhibited improved water solubility and a new thermal transition. It has been observed that the ring-opening polymerisation of ε-caprolactone onto phthaloyl-protected chitosan could be performed without the addition of any catalyst since it seems that the phthaloyl group works both as a protecting agent for the amino group and as a catalyst of the reaction [159]. In order to reduce the reaction time, microwave radiation can also be used to prepare chitosan/polycaprolactone graft copolymers [160].

Chitosan/oligo L-lactide graft copolymer has been synthesised in DMSO at 90ºC in the presence of $Ti(OBu)_4$ as ring opening catalyst [161]. The graft copolymers were converted to hydrogels on exposure to deionised water. At higher grafting percentages, the longer oligo L-lactide (OLLA) side chains have a tendency to self-assemble with each other by hydrogen bonding and dipole–dipole interactions between oligoester side chains, which results in the lower swelling of graft copolymers. Thermal stability and the melting transition temperature also increased at higher grafting percentages. A decrease in hydrolytic degradation was observed with increase of lactide content in the graft copolymer. Even though the graft copolymers were susceptible to both papain and lipase, the highly grafted chitosan was less susceptible to hydrolysis in papain medium whereas it was more prone to hydrolysis in lipase than the original chitosan and OLLA. These results indicate that the phys-

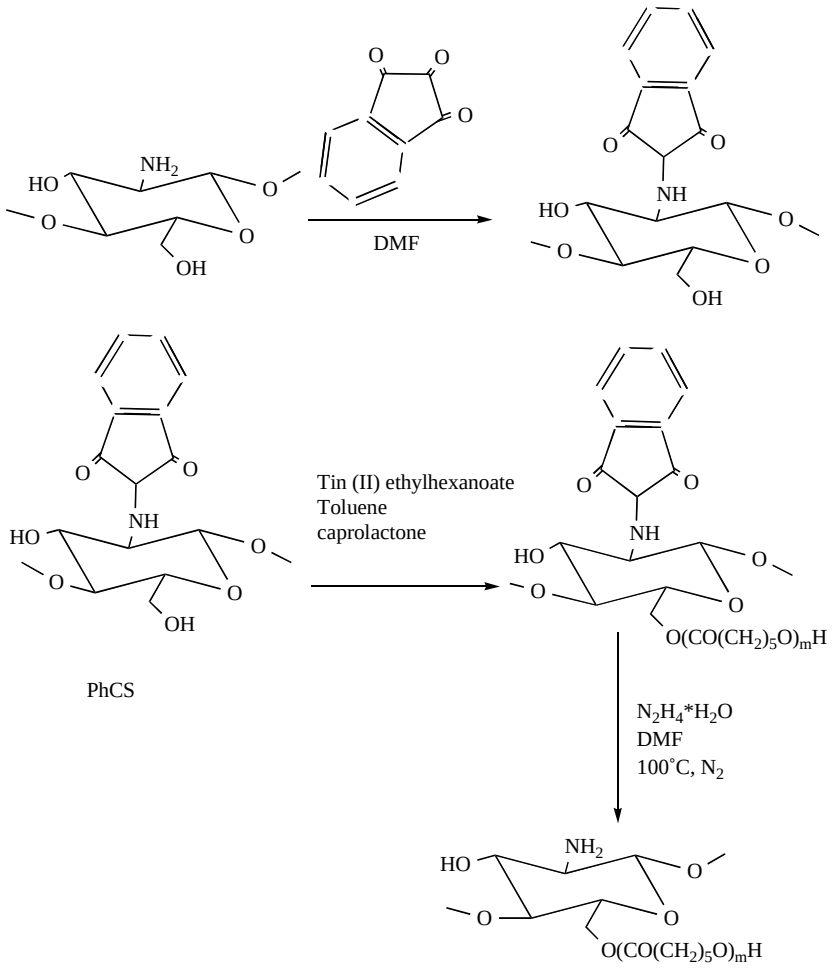

Fig. (12). Chitosan graft derivatives by opening ring polymerisation.

ico-chemical properties and the rate of degradation of graft copolymers as a biomaterial can be controlled by adjusting the amount of LLA in the graft copolymers which may find wide applications in wound dressing and in controlled drug delivery systems.

2.6.3. Raft Polymerisation

Raft polymerisation has been recently proposed to prepare chitosan graft derivatives [162]. A temperature-responsive chitosan has been synthesised by free radical polymerisation of N-isopropylacrylamide (NIPAM) at 60ºC in the presence of RAFT-chitosan agent [163]. The chitosan was modified with first phthalic anhydride to improve its solubility and subsequently modified with S-1-dodecyl-S' (αα'dimethyl-α'' acetic acid) trithiocarbonate to serve as reversible addition fragmentation chain transfer (RAFT) agent. The temperature played an important role on the self-assembly in H_2O dispersion and the morphologies of chitosan-g-PNIPAMs. At 40ºC chitosan-g-PNIPAMs in H_2O dispersion had the most symmetric size distributions, the smaller Z-average sizes and the narrower size distributions in comparison with the samples at 25ºC. TEM images showed the symmetric saturated spheres for the samples prepared at 25ºC, and irregular structures for the samples prepared at 40ºC. These results indicated that chitosan-g-PNIPAMs have a marked thermal responsive property.

Using the same methodology, acrilic acid has been grafted onto chitosan backbone (Fig. (**13**)) [164]. The size distribution of the self-assembly of chitosan-g-PAA in ethanol is very narrow, which may be associated with the grafting density and the "well-defined" PAAs onto chitosan from RAFT polymerisation. TEM shows that chitosan-g-PAA in diluted ethanol dispersions is roughly 80 nm in size with some aggregation. This work provides a new method to prepare chitosan grafting copolymers with controlled molecular weights and "well-defined" structures such as random copolymers, block polymers, and star-shaped polymers.

2.7. Miscellaneous Derivatives

2.7.1. Cyclodextrin Chitosan Derivatives

Cyclodextrins (CD) are cyclic oligosaccharides built from six to eight D-glucose units (α=6, β= 7, γ=8). The interest in these molecules is due to the ability of their hydrophobic cavity to bind small organic molecules including aromatic ones. A review covering the chemistry of cyclodextrins grafted to chitosan and the properties and applications of these derivatives as adsorbents has been published in 2006 [165]. Herein, we report the latest papers related to the preparation of chitosan derivatives with cyclodextrins.

Hydroxypropyl chitosan-graft-carboxymethyl β-cyclodextrin (HPCS-g-CM β-CD) has been synthesised by grafting CM β-CD onto HPCS using EDC as the condensing agent. The adsorption of a hydrophobic model drug, ketoprofen, by HPCS-g-CM β-CD microparticles (using tripolyphosphate as an ionic crosslinking agent) was carried out providing a slower release of the entrapped ketoprofen than chitosan. Moreover, release behaviour was influenced by

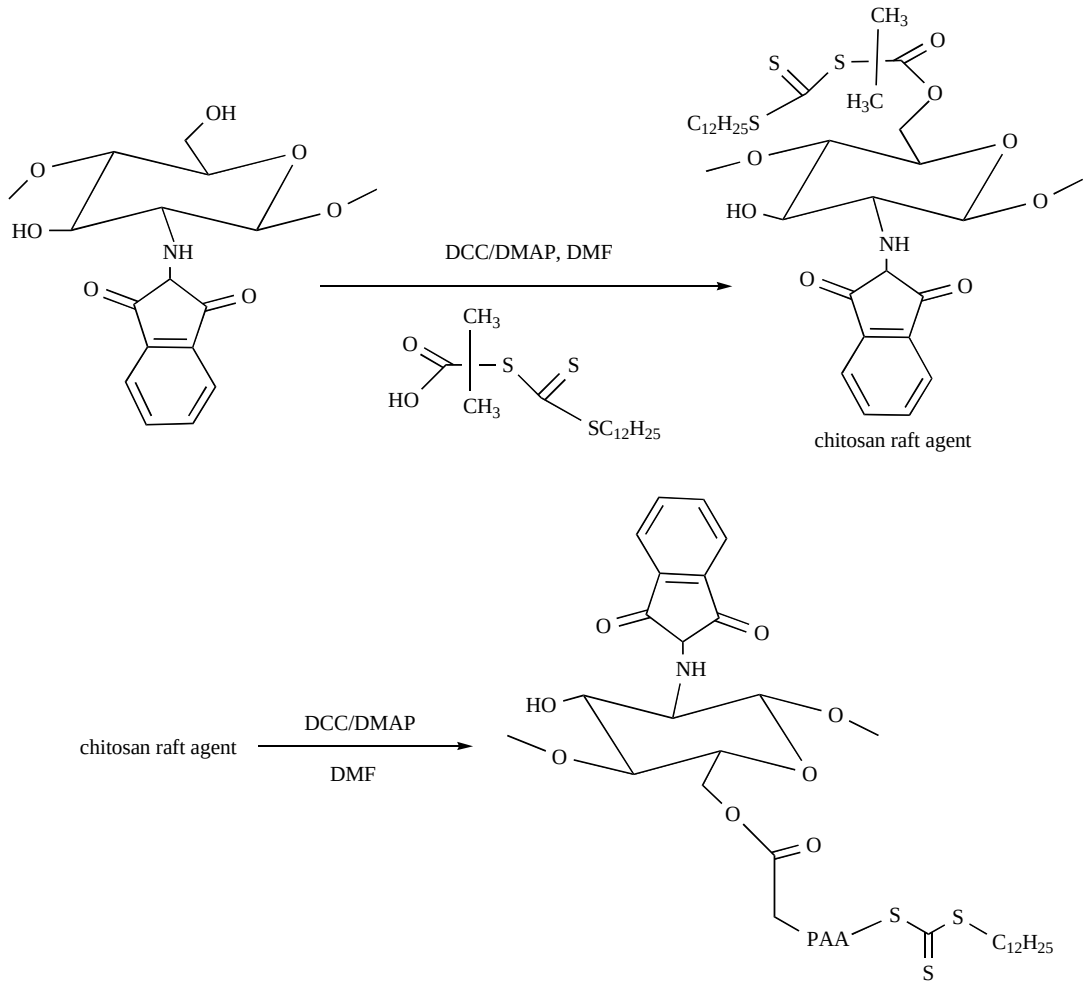

Fig. (13). Chitosan graft derivatives by raft polymerisation.

the pH value of the medium. These results suggest that β-CD grafted with chitosan derivatives may become a potential biodegradable delivery system for the controlled release of hydrophobic drugs with pH-responsive capability [166].

A novel thiolated carboxymethyl chitosan-g-β-cyclodextrin (CMC-g-β-CD) drug delivery carrier has been synthesised using two steps [167]. First, carboxymethyl β-cyclodextrin (CM β-CD) was grafted onto carboxymethyl chitosan using EDC and NHS as the condensing agents. Next, the resultant product was further grafted with cysteine methyl ester hydrochloride (CMEH) (Fig. (**14**)). The chitosan derivative showed higher swelling than chitosan. The adhesive properties of thiolated CMC-g-β-CD were also improved (fivefold increase in the adhesion time). Moreover, CMC-g-β-CD tablets provided a slower release of ketoprofen and the release behaviour was influenced by the amounts of thiol groups present on the polymer chains.

New β -cyclodextrin (β-CD) dimeric species have been synthesised in which the two CD moieties are connected by one or two hydrophilic oligo(ethylene oxide) spacers [168]. Their complexation with sodium adamantylacetate (free adamantane) and adamantane- grafted chitosan (AD-chitosan) was then studied and compared with their hydrophobic counterparts that contain an octamethylene spacer. Isothermal titration calorimetric experiments have demonstrated that the use of hydrophilic spacers between the two CDs instead of aliphatic chains makes almost all of the CD cavities available for the inclusion of free adamantane. Investigation of the interaction of the CDs with AD-chitosan by viscosity measurements strongly suggests that the molecular conformation of the CD dimeric species plays a crucial role in their cross-linking with the biopolymer. The derivative doubly linked with hydrophilic arms, also called a duplex, has been shown to be a more efficient cross-linking agent than its single bridged counterpart.

A chitosan-g-β-cyclodextrin derivative has been prepared as absorbent to remove phenol, p-nitrophenol and p-chlorophenol from aqueous solution [169]. Surprisingly, this derivative showed specific adsorption ability for p-chlorophenol. Separation of phenol and regeneration of the absorbent was carried out by simple washing with ethanol and filtrating.

2.7.2. Chitosan-PEG Derivatives

Polyethylene glycol (PEG) has been employed extensively in pharmaceutical and biomedical fields due to its physicochemical and biological properties including hydrophilic properties, water solubility, non-toxicity, biocompatibility, biodegradability, easy chemical modification and absence of antigenicity and immunogenicity.

Pegylation of chitosan has been carried out to prepare chitosan soluble derivatives that have subsequently been modified to introduce new properties for a wide range of applications (Table **8**). Targeting moieties such as folate [170] and galactose [171] have

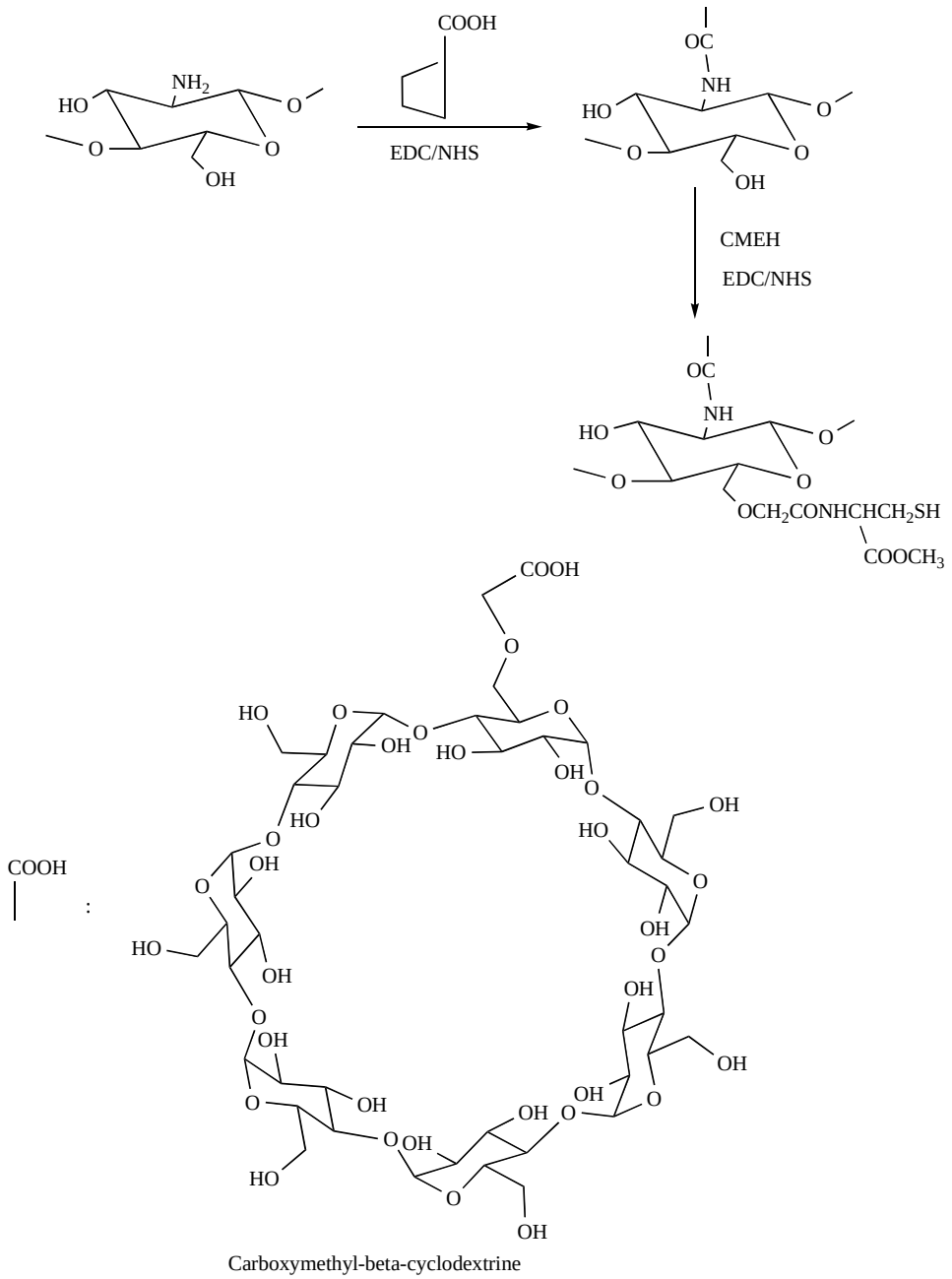

Fig. (14). Coupling of cyclodextrin to chitosan.

been linked to pegylated chitosan to improve gene transfection efficiency due to the promoted uptake of folate receptor bearing tumour cells and to improve receptor-mediated endocytosis on hepatocytes, respectively. Chitosan-g-PEI-g- PEG-OH possessing a hydroxyl group at the PEG chain end has been prepared by a grafting reaction between chitosan, polyethylenimine (PEI) and heterobifunctional PEG for constructing amphiphilic copolymers [172]. The amphiphilic and end-functionalised copolymer may have a potential utility in conjugating with targeting moieties for targetable gene or drug delivery.

Pegylated chitosan derivatives have been widely used in drug delivery. Caprolactone (PLC) has been linked directly to hydroxyl groups onto chitosan backbone or PEG hydroxyl groups to prepare amphiphilic polymers in a multi-step synthesis which includes protection/deprotection of the aminogroups of chitosan were necessary [173, 174].

The capability of N-phthaloylchitosan-grafted poly (ethylene glycol) methyl ether (mPEG)(PLC-g-mPEG) to enhance the aqueous solubility and stability of the lactone form of camptothecin (CPT) has been investigated [175]. Release of CPT from the micelles was sustained. When compared to the unprotected CPT, CPT-loaded PLC-g-mPEG micelles were able to prevent the hydrolysis of the lactone group of the drug ($t_{1/2}$= 94 min , $t_{1/2}$= 76 h, respectively).

A series of novel chitosan derivatives with octyl, sulfate and polyethylene glycol monomethyl ether (mPEG) groups as hydrophobic and hydrophilic moieties, respectively, have been synthesised [176]. Paclitaxel, was solubilised into the polymeric micelles

Table 8. Applications of Pegylated Chitosan Based Polymers

Application	Ref.
Cell targeting to improve gene delivery	[170-172]
Drug delivery	[173-181]
Tissue engineering	[182]
Blood compatible biomaterials	[183]

formed by these derivatives using a physical entrapment method, with micellar particle size around 100–130 nm. A paclitaxel concentration of 3.94 mg/ml was found in N-mPEG-N-octyl-O-sulfate chitosan (mPEGOSC) micellar solution, which was much higher than that in water (less than 0.001 mg/ml).

Amphiphilic chitosan developed by conjugating hydrophobic phthalimido groups and hydrophilic poly (ethylene glycol) chains gives a well-dispersed colloidal solution in polar solvents and shows a regular nano-sized spherical structure of 200 nm being the LD_{50} higher than 2000 mg/kg body weight [177]. The study on guest molecule incorporation using alkylamines and carboxylic acid as models suggests that the system may be useful for the release of hydrophobic and positively charged molecules.

Pegylated chitosan has been succesfully used to prepare polyion complex micelles (PIC micelles) to deliver anionic drugs such as diammonium glycyrrhizinate [178], Alltransretinoic acid [179], heparin [180] and retinoic acid [181]. PIC micelles are based on a inner core composed of an ion complex between a polymer (chitosan or chitosan derivatives in this particular case) and a drug, and an outer shell composed of hydrophilic domains such as MPEG.

Pegylated chitosan derivatives prepared by the esterification reaction between PEG and 6- O-succinate-N-phthaloyl-chitosan in the presence of water as a swelling agent have originated a porous net structure [182]. Since both chitosan and PEG are biocompatible and non-toxic this polymer is proposed to be used in tissue engineering

Chitosan based surfactant polymers that could be used to modify the surface of existing biomaterials in order to improve their blood compatibility have been developed from pegylated chitosan [183]. These polymers consist of a chitosan backbone, PEG side chains to repel non-specific protein adsorption, and hexanal side chains to facilitate adsorption and proper orientation onto a hydrophobic substrate via hydrophobic interactions. Since chitosan is a polycationic polymer, and it is thrombogenic, the surface charge was altered to determine the role of this charge in the hemocompatibility of chitosan. Charge had a notable effect on platelet adhesion. The platelet adhesion was greatest on the positively charged surface, and decreased by almost 50% with the neutralization of this charge. The inclusion of either negatively charged SO_3^- groups or a high density of large water-soluble PEG side chains produces a surface that may be suitable for cardiovascular applications.

3. EFFECT OF PHYSICO-CHEMICAL PROPERTIES (MW AND DD) ON CHITOSAN CHEMISTRY AND PROPERTIES OF THE AMPHIPHILIC DERIVATIVES.

Chitosan is a polymer with a high variability in its physicochemical properties due to its natural origin. Therefore, we considered that a study of these properties on the chemistry and applications of amphiphilic chitosan derivatives could be very useful. The first point to be noticed is that due to an inadequate characterisation of the chitosan samples used in the literature a direct comparison of results is not a trivial issue. Therefore, we have concentrated our efforts in those articles that study the effect of different parameters such as molecular weight (Mw) and deacetylation degree (DD) of chitosan.

Typically, chitosan samples with a Mw of around 150-200 kDa are used to prepare N-akyl chitosan derivatives for rheological studies. do Santos *et al* have studied the rheological properties of alkyl-chitosan derivatives using a low Mw chitosan (35 kDa) [184]. A non-Newtonian behaviour due to the presence of hydrophobic groups was observed. The solution viscosity at higher polymer concentration increased, but decreased at higher temperatures, which is in good agreement with previously results using samples of higher molecular weight.

Zhang *et al.* have observed that the degree of substitution of N-octyl N, O Carboxymethyl chitosan depended on the molecular weight of the chitosan sample (5, 10, 20 kDa). However, a clear tendency was not observed. The size and CMC of the micelles was slightly affected by the Mw of chitosan. The lower Mw sample had the lower CMC and the largest size [185]. The *in vitro* cytotoxic effect of the PTX-loaded micelles was comparable to that of the commercial formulation Taxol, which was not affected by the Mw of chitosan

When studying the effect of chitosan Mw (20, 38 ,300 and 1100 kDa) on the properties of oleoyl-chitosan it has been observed that the Mw affected both the viscosity and morphology of the samples [186]. At low Mw, spherical nanoparticles were observed but at high Mw no nanoparticles were observed probably due to the high viscosity of the derivative.

The morphology of caboxyethyl chitosan also depended on the Mw of chitosan [187]. At 45 kDa only spherical morphologies were observed regardless the pH and polymer concentration studied. On the other hand, chitosan derivatives with a chitosan Mw of 670 kDa showed spherical morphologies at pH 5.0 and 5.6 while at pH 5.2 a spherical morphology was only observed at low polymer concentration. On increasing polymer concentration rod and worm-like structures were observed.

The effect of deacetylation degree on the properties of N-phthaloylchitosan-g-mPEG (PhCS-g-mPEG) has been studied by Opanasopit [188]. The CMC of nanoparticles with different DD in water was similar (28 g/ml) but the size of the micelles increased with DD. The cytotoxicity of phthaloylchitosans (PhCS) and PLC-g-mPEG in Hela cell line was evaluated. The results showed that cytotoxicity increased with increasing DD of chitosan, being the cytotoxicity of PhCS-g-mPEG significantly lower than that of PhCS. Camptothecin, as a model drug, was loaded into the inner core of the micelles by a dialysis method. It was found that % DD of chitosan, corresponding to the N-phthaloyl groups in the inner core of the nanoparticle obtained, was a key factor in controlling the yield, the stability of the drug-loaded micelles, and the drug release behaviour. As the % DD increased, the CPT-loaded micelles

Table 9. Applications of Amphiphilic Chitosan Derivatives

Biomedicine/pharmacy	Smart polymers Drug delivery (mainly hydrophobic drugs) Gene delivery Cell targeting Tissue engineering Antibacterial coatings Blood compatible materials Biolabeling Biosensors
Technology	Fuel cell anode Artificial viscosifiers (paints, food industry) Dispersant Stabilisation of nanoparticles Sensors Waste-water treatment Encapsulation of neutraceuticals/cosmetics

stability increased. Release of CPT from the micelles was dependent on the %DD; a sustained release was obtained with %DD. All-trans retinoic acid (ATRA) has also been incorporated into PLC-g-mPEG by dialysis method in an attempt to optimize carriers for ATRA delivery [189]. A similar behaviour regarding DD was observed. When compared to the unprotected ATRA, ATRA loaded in PLC-g-mPEG micelles was efficiently protected from photodegradation.

4. CONCLUSIONS

Chitosan is a versatile polymer with a wide range of applications and properties. Due to the presence of reactive groups (NH_2, OH) on its backbone, chitosan can be easily modified to improve its properties or to open the use of chitosan in new applications. A summary of the applications of amphiphilic chitosan derivatives is shown in Table **9**. The introduction of a hydrophobic molecule on the chitosan backbone modified the rheological behaviour of the polymer being these derivatives used as rheological modifiers in the food, cosmetic or pharmaceutical industries. The ability of amphiphilic derivatives to self-assemble has a great interest in the field of biomedicine. Micellar systems based on chitosan derivatives have been used as drug delivery systems, mainly for hydrophobic drugs but also for anionic drugs and proteins. Amphiphilic chitosan derivatives have also been proved very useful in gene therapy since it seems that the presence of hydrophobic moieties on the chitosan backbone improves the transfection when compared with chitosan. Some amphiphilic chitosan derivatives have also exhibited interesting biological properties such as antibacterial activity, blood compatibility, mucoadhesivity and the ability to open tight junctions. Moreover, some amphiphilic derivatives are smart materials whose behaviour is affected by pH and /or temperature.

Amphiphilic chitosan derivatives are widely proposed for their use in pharmacy and biomedicine, but neither chitosan nor its derivatives have yet been approved by the FDA. Studies regarding the biocompatibility and toxicity of amphiphilic chitosan derivatives have been carried out and the results seem to show the biocompatibility and non-toxicity of these derivatives. The LD_{50} value of N-octyl-O-sulfate chitosan administrated by i.v. and i.p. were calculated as 102.59 and 130.53 mg/kg, respectively while LD50 value of PEG-g-phtaloylchitosan was 2000 mg/kg. However, to our knowledge no long term studies of human safety have been reported.

It seems that chitosan Mw affects the chemistry (degree of substitution) and morphology of the self-assembly derivatives. Moreover, chitosan DD also affects the behaviour of amphiphilic chitosan derivatives in drug delivery. Surprisingly, this fact is not taken into account in the literature and in general, a lack of chitosan characterisation is observed.

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