CHAPTER 2
LITERATURE REVIEWS

2.1 Chitosan

Chitosan is a cationic biopolymer, one of the most important partially deacetylated derivatives obtained from chitin by chemical or enzymic reaction, which is the second abundant polysaccharide next to cellulose in nature. Chitin is found in the exoskeleton of insects, crustaceans such as crabs, shrimps, lobsters, squid pens and the cell walls of some fungi such as *Zygomicetes*, *Aspergillus* and *Mucor* [5]. However, the utilization of chitin has been restricted by its intractability and insolubility, because it is highly hydrophobic and insoluble in water and most organic solvents. Chitin is soluble in hexafluoroisopropanol, hexafluoroacetone, chloroalcohols in conjunction with aqueous solutions of mineral acids, thus chitosan is considerably more versatile than chitin [2].

2.1.1 Chemical structure of chitosan

Chitosan (2-amino-2-deoxy-(1-4)-β-D-glucopyranan) is a copolymer consists of D-glucosamine and N-acetyl-D-glucosamine units, randomly or block distributed throughout the biopolymer. It structure very similar to cellulose and chitin, except that the amino group replaces the hydroxyl group and acetylamide group on the C-2 position of the glucose rings [32]. The structural details of cellulose, chitin and chitosan are shown in Figure 1. The characteristics of chitosan that consist of degree of N-deacetylation (40-98%), defined in terms of the percentage of primary amino groups in the polymer backbone, and average molecular weights (50-2,000 kDa) are very important to the physicochemical properties of chitosan utilization and they have been a major effect on the biological properties [6, 7, 33].

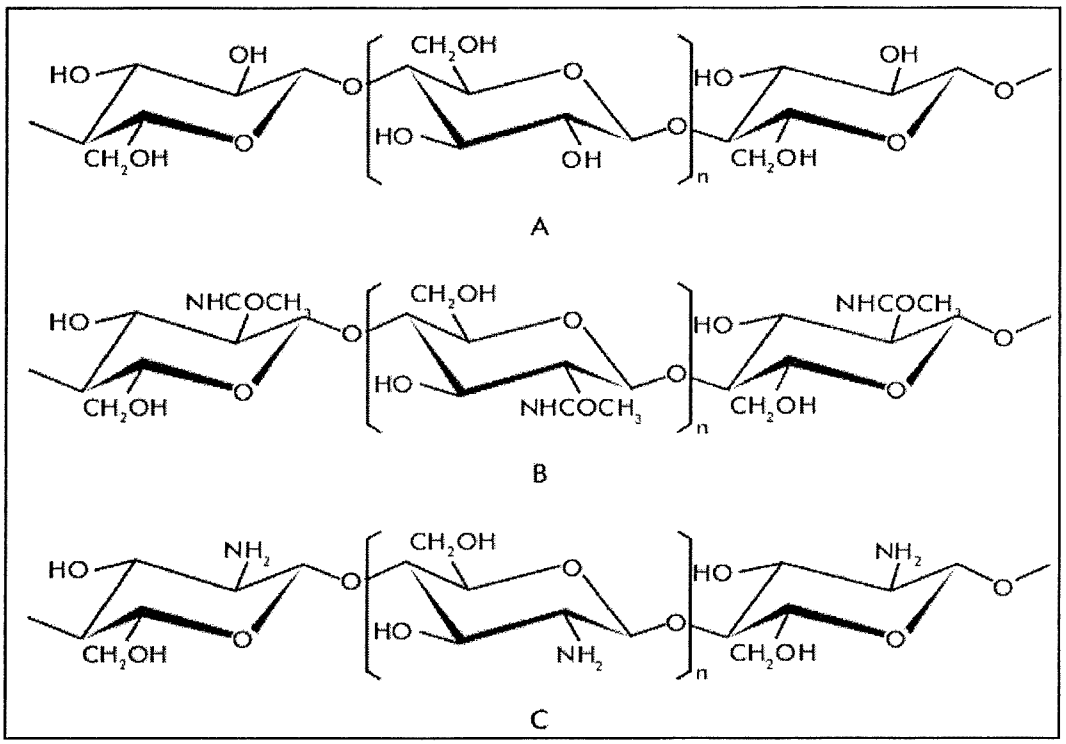

Figure 1 Structural representation (A) cellulose, (B) fully acetylated chitin and (C) fully deacetylated chitosan, evidencing their structural similarity.

Source: Ramírez, M. A. et al., (2010). "Chitin and its derivatives as biopolymers with potential agricultural applications." **Biotecnología Aplicada** 27: 271.

2.1.2 Properties of chitosan

Most of the naturally occurring polysaccharides such as cellulose, pectin, alginic acid, agar, agarose and carragenas are natural and acidic in nature. Meanwhile, chitin and chitosan are examples of highly basic polysaccharides. Their properties include solubility in various media, solution, viscosity, polyelectrolyte behavior, and ability of form films, metal chelations and structural characteristics [34]. The chemical and biological properties of chitosan were reported in Tables 1 and 2 [6]. These properties make chitosan as a good candidate for the development of conventional and novel drug and gene delivery systems [35]. Chitosan acetate was combined with hydroxypropyl methylcellulose, showed the potential of a new combination coating material for colonic drug delivery [10]. It has also been used as a pharmaceutical excipient, as a diluents compression of tablets and a binder in wet granulation [15]. Chitosan performs as an absorption enhancer in the intestine by

increasing the residence time of dosage forms at mucosal sites, inhibiting proteolytic enzymes and increasing the permeability of protein and peptide drugs across mucosal membranes. Chitosan is degraded by the microflora that is available in the colon. As a result, this compound could be promising for colon-specific drug delivery [36]. Chitosan esters, such as chitosan succinate and chitosan phthalate have been used successfully as potential matrices for the colon-specific oral delivery [31].

Film forming property of chitosan

Chitosan has been well known for its good film-forming property as a result of intra- and intermolecular hydrogen bonding. Films from aqueous acidic chitosan solution are clear, tough, flexible and good oxygen barriers [37]. Films prepared from pure polymers tend to be brittle and often crack upon drying. Addition of food-grade plasticizers to film-forming solution alleviates this problem [38]. A number of researches report the preparation of chitosan derivative films and study of mechanical and physical properties. For example, the alkyl-chitosan derivatives appear to be more plastic than chitosan films but less resistant [39]. N, N, N,-Trimethyl chitosan demonstrated a good film forming ability and a significant reduction in contact angle corresponds to an increase in the degree of hydrophilicity [40]. Carboxymethyl chitosan film was reported that the vapor permeability value was higher than chitosan film [41]. Moreover, chitosan films can be used in widely applications such as materials in food packaging, antimicrobial packaging, coating and wound dressing as well as drug release membranes [42-45]. Although, chitosan has been investigated for colon-specific delivery of drugs because of its biodegradability by colonic bacteria [46], the application of chitosan as enteric coating was less successfully due to its readily dissolving in acidic conditions [47].

Table 1 Chemical properties of chitosan.

Chemical properties of chitosan
Cationic polyamine
High charge density at pH<6.5
Adheres to negatively charged surfaces
Film-forming ability
Forms gels with polyanions
High molecular weight linear polyelectrolyte
Viscosity, high to low
Chelates certain transitional metals
Amiable to chemical modification
Reactive amino/ hydroxyl groups

Table 2 Biological properties of chitosan.

Biological properties of chitosan
Biocompatibility
Natural polymer
Biodegradable to normal body constituents
Safe and non-toxic
Hemostatic, bacteriostatic and fungstatic
Spermicidal
Anticancerogen
Anticholesteremic
Reasonable cost
Versatile

Source: Adapt from Hejazi, R. and M. Amiji, (2003). "Chitosan-based gastrointestinal delivery systems." **Journal of Controlled Release** 89: 153-154.

2.1.3 Solubility of chitosan

Chitosan is soluble only in acidic media and part inorganic acid, such as acetic acid, citric acid, malic acid and hydrochloric acid [15], due to the presence of free amino groups along the polymer chains make chitosan is a cationic polyelectrolyte ($pK_a \approx$ 6.5). It is normally insoluble in water, alkali or aqueous solutions above pH 6.5 and organic solvents make its utilization limited in the pharmaceutical field as protein delivery is the easy dissolution of chitosan in the low pH of stomach [33]. Because its semi-crystalline structure, which is attributed to extensive intramolecular and intermolecular hydrogen bonding between the chains and sheets, respectively (Figure 2) [12-14]. Despite this limitation, various applications of chitosan and modified chitosan have been reported. Hence, improving the solubility of chitosan is important if this abundant resource is to be utilized across a wide pH range [49].

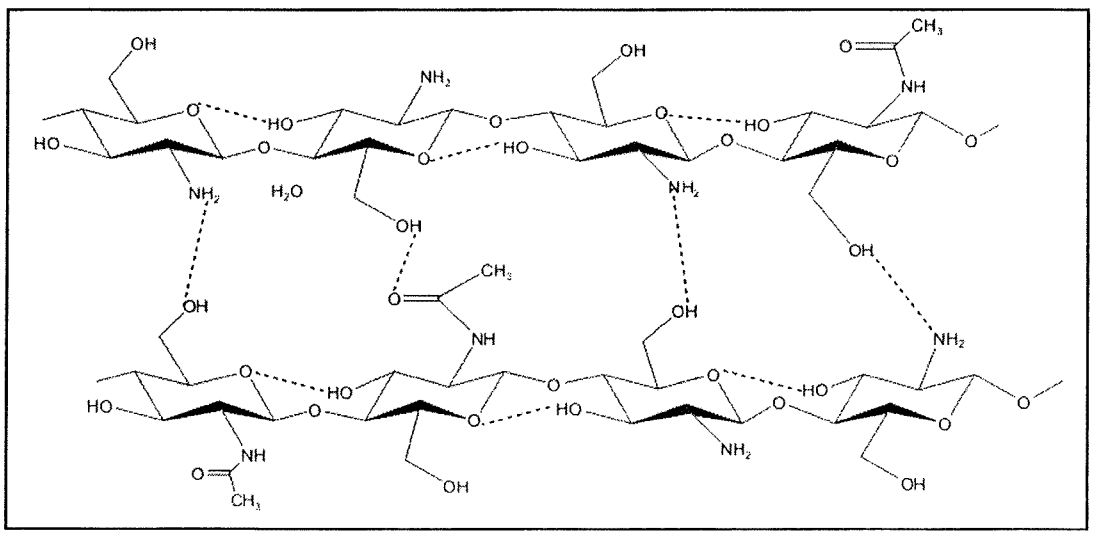

Figure 2 Crystalline structure of chitosan.

Source: Champagne, L. M. (2008). "The synthesis of water soluble / n-acyl chitosan derivatives for characterization as antibacterial agents." PhD thesis, Chemistry, Louisiana State University and Agricultural & Mechanical College: 14.

2.2 Chemical modifications of chitosan

In order to improve or impart new properties to chitosan, chemical modification of chitosan is important and necessary. The chemical modification of chitosan is interesting because it not only unchanged the fundamental skeleton of chitosan but also keep the original physicochemical and biochemical properties and finally could bring new or improved properties [21]. Chitosan chains compose of three attractive reactive sites for chemical modification as two hydroxyl groups (a primary hydroxyl at C-6 and a secondary hydroxyl at C-3) and a highly reactive amino group (at C-2) (Figure 3) [13]. The site of modification is dictated by the desired application of the final chitosan derivative [4]. The scope for preparing a lot of derivatives are shown in Figure 4 [13]. The presence of free amine groups along the chitosan chain, which could be suitably modified by various chemical reactions to impart desired properties and enhance solubility in water as well as in organic solvents [32, 50]. The amino functionality gives starting chemical reactions such as alkylation, acylation, quaternization, hydroxyalkylation, carboxyalkylation, thiolation, sulfation, phosphorylation, graft copolymerization, etc [14, 24, 40, 51-53]. Moreover, the derivatives provided a variety of products with properties such as antibacterial, anti-fungal, anti-viral, anti-acid, anti-ulcer, non-toxic, non-allergenic, total biocompatibility and biodegradability, etc [32, 54, 55].

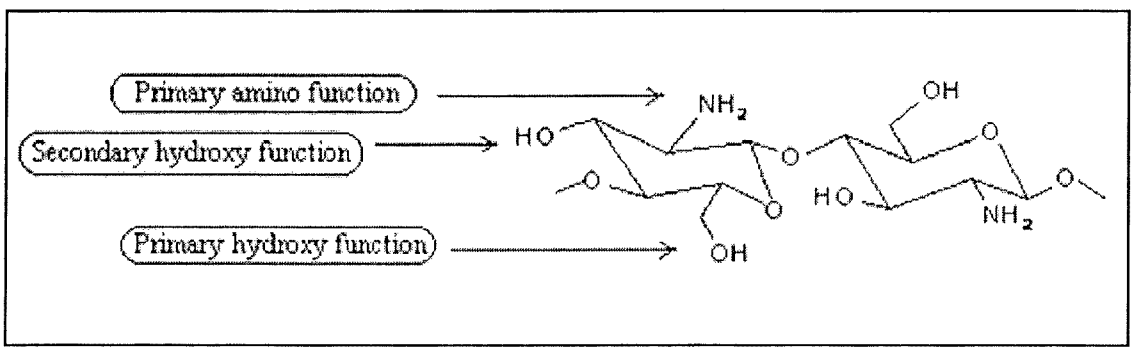

Figure 3 Functional groups of chitosan.

Source: Mourya, V.K., and N. N. Inamdar. (2008) "Chitosan-modifications and applications: Opportunities galore." **Reactive & Functional Polymers** 68: 1017.

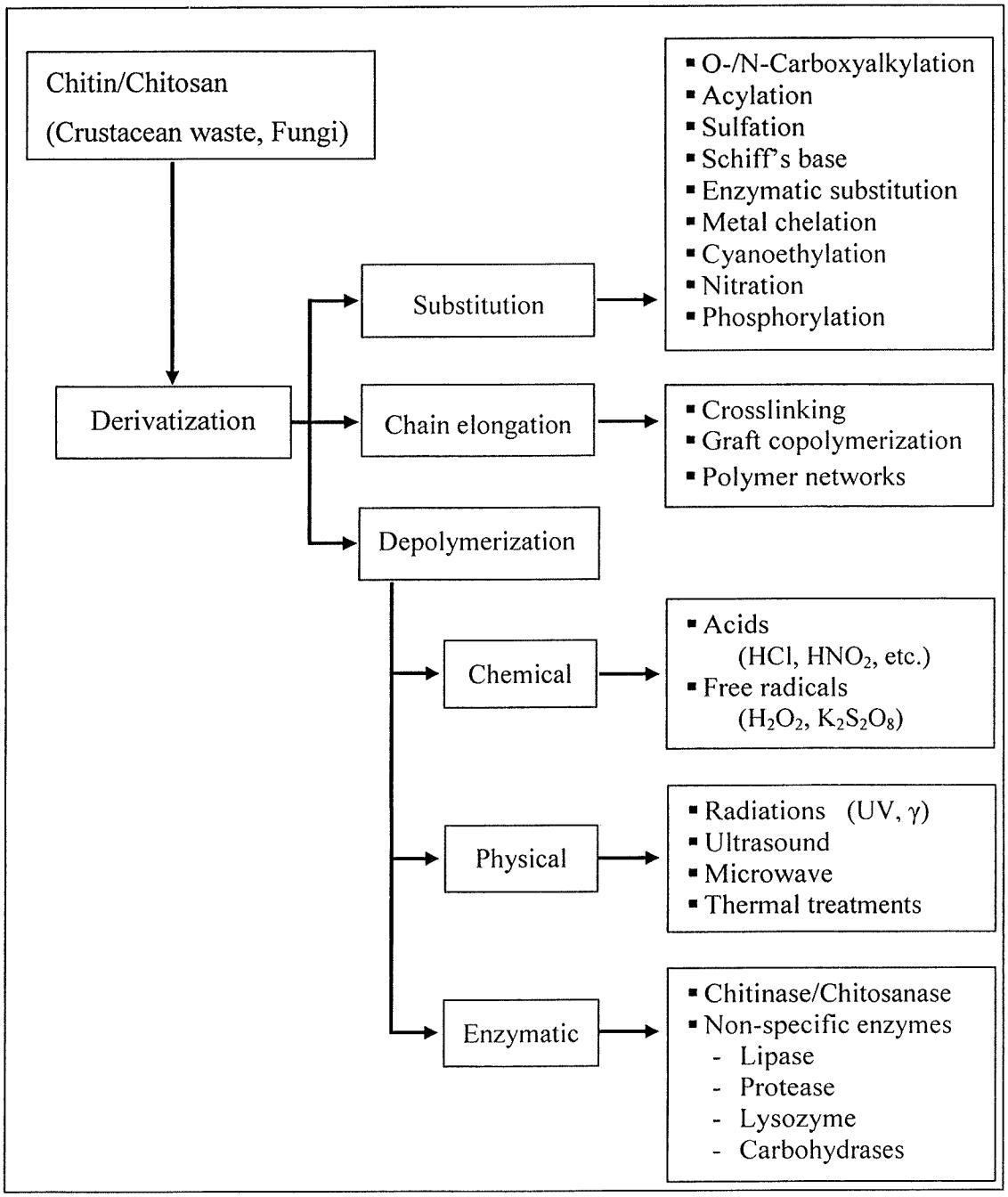

Figure 4 Multifaceted derivatization potential of chitin and chitosan.

Source: Adapt from Prashanth, K.V.H. and R.N. Tharanathan, (2007). "Chitin/ chitosan: modifications and their unlimited application potential-an overview." **Trends in Food Science & Technology**, 18: 119.

2.2.1 N-Acylation chitosan

N-Acyl derivatives of chitosan are the most typical and extensively studied modification reaction. Since, chitosan is a strong nucleophile because of the presence of nonbonding pair of electrons on its primary amino groups [56]. Chitosan reacts readily with most aldehydes to produce imines. It also reacts with acid anhydride and acyl halide to form the corresponding N-Acyl derivatives (Figure 5) [21, 57].

N-acylation of chitosan has been achieved with various kinds of acid anhydride. Since most acid anhydrides exhibit very little solubility in aqueous media, the reactions between chitosan and acid anhydrides are conducted under heterogeneous experimental conditions. In a general way, acylation reactions lead frequently in mediums as aqueous acetic acid/methanol, pyridine/chloroform, trichloroacetic acid/dichloroethane, ethanol/methanol mixture, methanol/formamide or DMA-LiCl [23]. N-acylation with acetic anhydride was reported to give an improved method of preparing water-soluble chitosan. The haft N-acetylated chitosan exhibited good water solubility. Thus, the amount of acetic anhydride was the most important factor affecting the substitution degree of the chitosan [58].

2.2.2 Cyclic acid anhydride

Acid anhydrides are formed from the dehydration reaction of two carboxylic acid groups. Anhydrides are highly reactive toward nucleophiles and are able to acylate a number of the important functional groups of proteins and other macromolecules as well as amino groups of chitosan [57]. Cyclic acid anhydrides are used for acylation reaction of chitosan via ring-opening reaction giving N-acyl chitosans, e.g. succinic, maleic, glutaric, itaconic and phthalic anhydrides, this reaction is easily to prepare under mind condition [23, 24].

N-acylation via ring opening reactions of partially deacetylated chitosan (88% DD) with various cyclic anhydrides in aqueous methanol system was reported [24]. The authors reported that all of the derivatives (DS = 0.21-0.80) displayed solubility in water at various pH. All of the products exhibited solubility in the pH region below 4.0 and above pH 7.0 (Table 3). The solubility in the acidic

region would be caused by the protonation of the N-amino groups ($-NH_2$ to $-NH_3^+$), and the solubility in the basic region would be caused by the change of the carboxy groups to carboxylate ions (–COOH to $-COO^-$). The derivatives did not exhibit complete solubility in the pH range of 4.0 – 7.0, which corresponded to the isoelectric point of the products. In this pH range, an equimolar of $-NH_3^+$ and $-COO^-$ groups existed in the macromolecule [24].

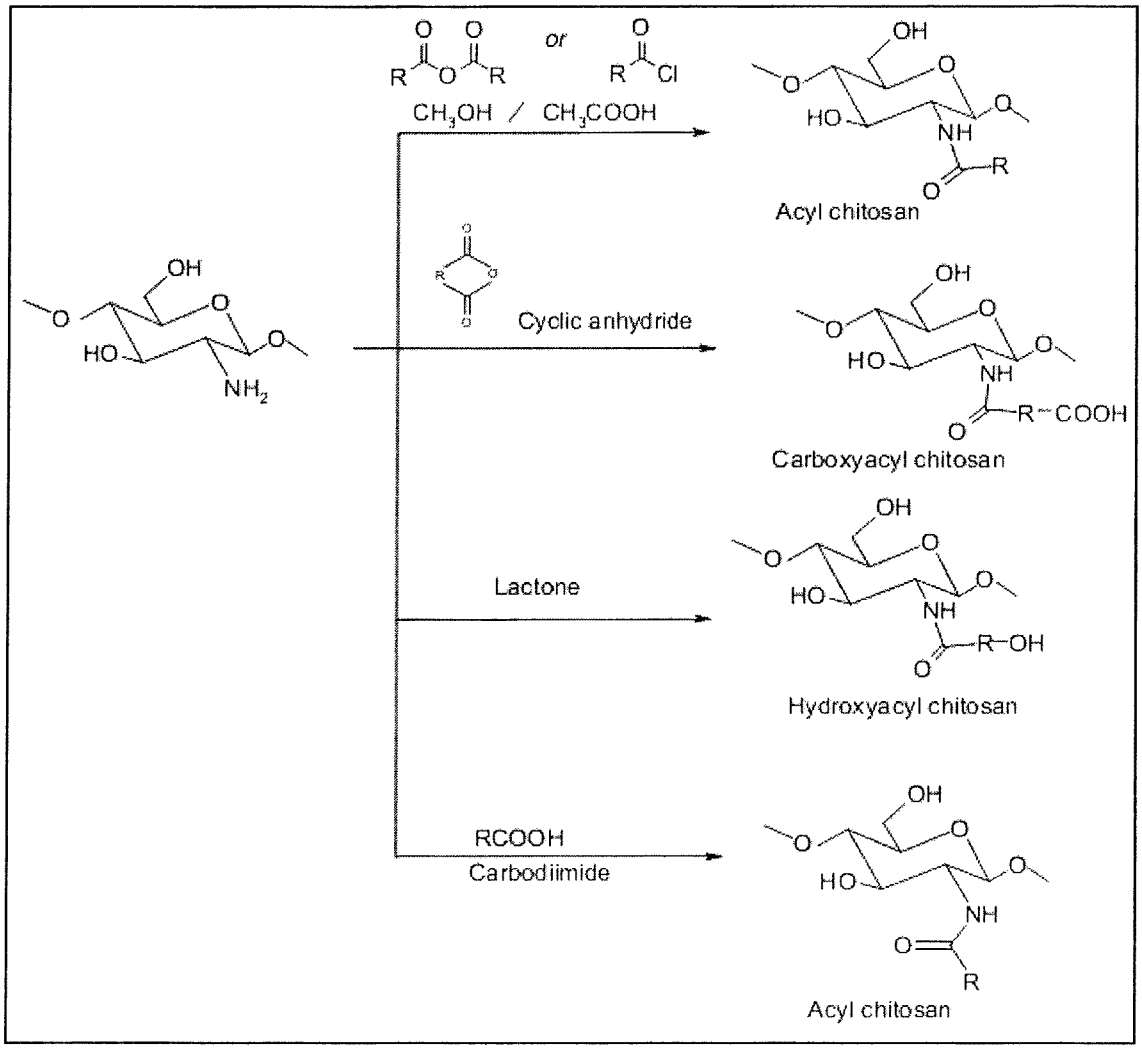

Figure 5 Acylation of chitosan.

Source: Mourya, V.K. and N. N. Inamdar. (2008) "Chitosan-modifications and applications: Opportunities galore." **Reactive & Functional Polymers** 68: 1026.

Table 3 Solubility of N-acylated chitosan (88%DD) in water of various pH[a].

Sample[b]	DS	Solubility[c] pH: 1 3 5 7 9 11 13
Suc	0.32	
Gltl	0.43	
Phth	0.34	
THP	0.32	
Norb	0.33	
Cycl	0.36	
Trim	0.20	
CS-88		

a. Solid sample (100 mg; 88%DD) was dispersed in H_2O (20 mL). The pH of the solution was adjusted with 0.5% (w/v) aqueous HCl and NaOH.

b. Suc, succinic; Gltl, glutalic; Phth, phthalic; THP, cis-1,2,3,6-tetrahydrophthalic; Norb, 5-norbornyl-endo-2,3-dicarboxylic; Cycl, cis-1,2-cyclohexyl dicarboxylic; Trim, trimellitic anhydride; CS-88, chitosan (88%DD).

c. white bar, soluble; black bar, insoluble.

Source: Adapt from Sashiwa, H. and Y. Shigemasa, (1999). "Chemical modification of chitin and chitosan 2: preparation and water soluble property of N-acylated or N-alkylated partially deacetylated chitins." **Carbohydrate Polymers** 39: 129.

Succinic anhydride

N-succinyl chitosan has been reports by many researchers. It has been obtained via ring-opening reaction by introduction of succinyl groups into N-terminal of the glucosamine units of chitosan (Figure 6) [28]. Succinylation degree of N-succinyl chitosan could be easily modified by changing reaction conditions using succinic anhydride in various solvent systems, such as acetic acid/ethanol, methanol, acetone, dimethylsulfoxide (DMSO) and dimethylformamide (DMF). The reaction temperature ranged from room temperature to 65°C for 3-24 h. Moreover, chitosan

was used in thcir reactions have the ranging of deacetylation degree from 85 to 90 %DD and molecular weight ranging from10 to 300 kDa [26-30, 59]. The structure changes of derivatives were confirmed by ^{1}H-NMR, ^{13}C-NMR and FTIR spectroscopy and their physical properties were analyzed by powder X-ray diffraction (PXRD) and differential scanning calorimetry (DSC) to investigate the crystallinity of the derivatives. The comparison between the FTIR spectra and the PXRD patterns of chitosan and N-succinyl chitosan were reported (Figure 7-8). In the FTIR spectrum of N-succinyl chitosan, the absorption bands of stretching vibration of the –OH and –NH_3 (3300–3500 cm^{-1}) became narrower and shifted to lower wave number after introducing succinyl groups. Another major change could be observed at the new absorption band of 1413 cm^{-1}, corresponding to symmetric stretching of the –COO group. The peak at 1650 cm^{-1} (Amide I) increased and no absorption bands appeared at 1720–1750 cm^{-1}, indicating that the succinyl derivation reaction took place at the N-position and –NH–CO– groups have been formed. Meanwhile, the peak at 1597 cm^{-1} (–NH_2 bending) decreased greatly, and the new signal at 1566 cm^{-1} (assigned to the secondary amines) further suggested that the amino groups of chitosan were substituted. The X-ray diffraction pattern of chitosan showed distinct crystalline peaks at around 11° and 20° compared to N-succinyl chitosan. A lot of strong intermolecular and intramolecular hydrogen bonds (H- bonds) make chitosan to form crystalline regions easily and be insoluble in water. However, the peak at 11° in N-succinyl chitosan X-ray diffraction spectrum disappeared and the peak at 20° weakened obviously. This result suggests that with the substitution of N-succinyl resulting in the hydrogen bonding capacity decreased. The solubility of N-succinyl chitosan in various pH was reported, it has degree of substitution of 33% showed the solubility of both acidic and basic region (Figure 9). As the derivative has both amino and carboxy group, the solubility in acid region (pH 1-3) would be caused by the protonation of amino group. The solubility in alkaline region (pH 7-13) would also be caused by the change of carboxy group to carboxylate ion. In addition, the solubility between pH 4.5-6.8 would be owing to the isoelectric point which exists equimolar of -NH_3^+ and -COO^- groups in the molecule [30]. Whereas N-succinyl chitosan with high degree of substitution (degree of substitution > 65%) exhibits the

opposite behavior of chitosan [21]. The application of N-succinyl chitosan has been widely researched in cosmetics and pharmaceutical field such as drug carrier, film or membrane formation, enzyme immobilization [25, 29, 59]. However, it was initially developed as wound dressing materials, it is currently also applied as cosmetic materials (Moistfine liquids) [59].

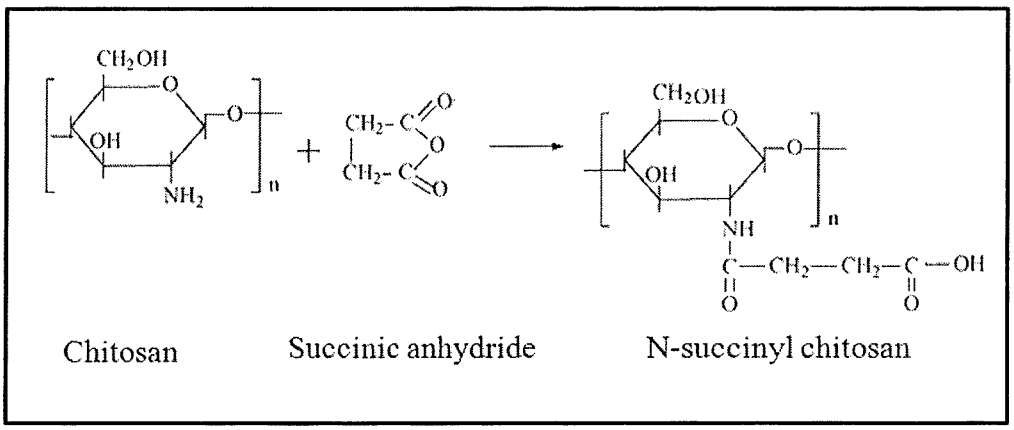

Figure 6 Modification of N-succinyl chitosan.

Source: Sui, W. et al. (2008). "Preparation and properties of an amphiphilic derivative of succinyl-chitosan." **Colloids and Surfaces A: Physicochem. Eng. Aspects** 316: 172.

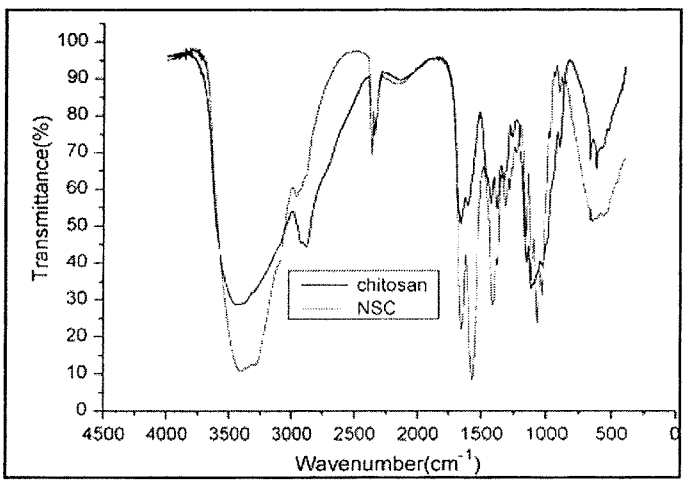

Figure 7 FTIR spectra of chitosan and N-succinyl chitosan (NSC).

Source: Zhou, J. Q. and J. W. Wang (2009). "Immobilization of alliinase with a water soluble–insoluble reversible N-succinyl-chitosan for allicin production" **Enzyme and Microbial Technology** 45: 301.

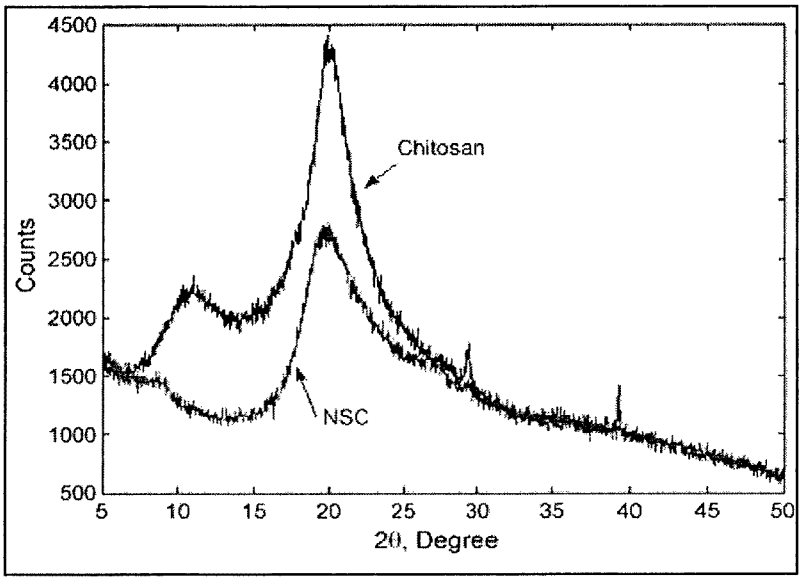

Figure 8 The X-ray diffraction patterns of chitosan and N-succinyl chitosan (NSC). Source: Zhou, J. Q. and J. W. Wang (2009). "Immobilization of alliinase with a water soluble–insoluble reversible N-succinyl-chitosan for allicin production" **Enzyme and Microbial Technology** 45: 301.

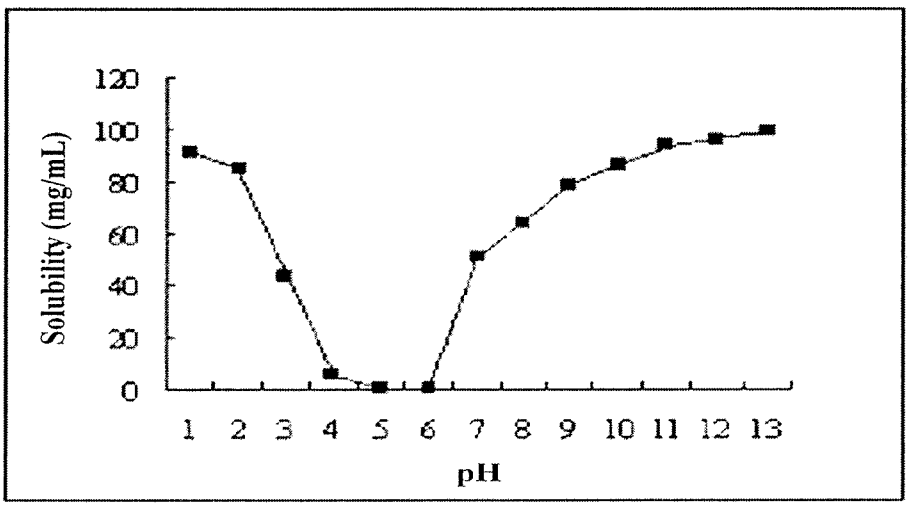

Figure 9 Solubility of N-succinyl chitosan (DS=0.33) in water of various pH. Source: Yan, C., et al. (2006). "Preparation of N-succinyl chitosan and their physical-chemical properties as a novel excipient." **Yakugaku Zasshi: Journal of the Pharmaceutical Society of Japan** 126 (9): 791.

Phthalic anhydride

Phthalic anhydride is a member of cyclic acid anhydrides that utilized in this study by introduction of phthaloyl groups into amino groups of the glucosamine units of chitosan. Phthalic anhydride itself is used as a monomer for synthetic resins such as glyptal, the alkyd resins and polyester resins [60]. Phthalates, in combination with various polymers, may be used as plasticizers and film coating agents in orally ingested solid pharmaceutical dosage forms and in numerous types of modified-release drug delivery systems such as enteric-coated and delayed-release tablets, pelletized delayed-release capsules, enteric-coated capsules, and controlled-release transdermal films [61]. Several different phthalates are currently used as excipients in approved pharmaceutical formulations:

1. Cellulose acetate phthalate (CAP)

CAP is produced by reacting the partial acetate ester of cellulose with phthalic anhydride in the presence of a tertiary organic base such as pyridine, or a strong acid such as sulfuric acid. CAP is used as an enteric film coating material, or as a matrix binder for tablets and capsules. Such coatings resist prolonged contact with the strongly acidic gastric fluid, but dissolve in the mildly acidic or neutral intestinal environment. CAP is commonly applied to solid-dosage forms either by coating from organic or aqueous solvent systems, or by direct compression. Concentrations generally used are 0.5–9.0% of the core weight. The addition of plasticizers improves the water resistance of this coating material, and formulations using such plasticizers are more effective than when CAP is used alone [62].

2. Dibutyl Phthalate (DBP)

DBP is produced from n-butanol and phthalic anhydride in an ester formation reaction. DBP is used in pharmaceutical formulations as a plasticizer in film-coatings. It has been evaluated as a pore-forming agent in novel delivery systems. It is also used extensively as a solvent, particularly in cosmetic formulations such as antiperspirants, hair shampoos, and hair sprays [63].

3. Diethyl Phthalate (DEP)

DEP is produced by the reaction of phthalic anhydride with ethanol in the presence of sulfuric acid. It is used as a plasticizer for film coatings on tablets,

beads, and granules at concentrations of 10–30% by weight of polymer. DEP is also used as an alcohol denaturant and as a solvent for cellulose acetate in the manufacture of varnishes and dopes [64].

4. Dimethyl Phthalate (DMP)

DMP is produced industrially from phthalic anhydride and methanol. DMP is used in pharmaceutical applications as a solvent and plasticizer for film-coatings such as hydroxypropyl methylcellulose, cellulose acetate and cellulose acetate–butyrate mixtures. In addition to a number of industrial applications, DMP is also widely used as an insect repellent with topical preparations typically applied as a 40% cream or lotion.

5. Hydroxypropyl methylcellulose phthalate (HPMCP)

HPMCP is prepared by the esterification of hypromellose with phthalic anhydride. The degree of alkyloxy and carboxybenzoyl substitution determines the properties of the polymer and in particular the pH at which it dissolves in aqueous media. It is widely used in oral pharmaceutical formulations as an enteric coating material for tablets or granules. HPMCP is insoluble in gastric fluid but will swell and dissolve rapidly in the upper intestine. Generally, concentrations of 5–10% of HPMCP are employed with the material being dissolved in either a dichloromethane : ethanol (50 : 50) or an ethanol : water (80 : 20) solvent mixture. HPMCP can normally be applied to tablets and granules without the addition of a plasticizer or other film formers, using established coating techniques [65].

6. Polyvinyl Acetate Phthalate (PVAP)

Polyvinyl acetate phthalate is a reaction product of phthalic anhydride, sodium acetate, and a partially hydrolyzed polyvinyl alcohol. The polyvinyl alcohol is a low molecular weight grade, and 87–89 mole percent is hydrolyzed. Therefore, the polyvinyl acetate phthalate polymer is a partial esterification of partially hydrolyzed polyvinyl acetate. PVAP is a viscosity-modifying agent that is used in pharmaceutical formulations to produce enteric coatings for products and for the core sealing of tablets prior to a sugar-coating process. PVAP does not exhibit tackiness during coating and produces strong robust

films. Plasticizers are often included in PVAP coating formulations to enable a continuous, homogeneous, noncracking film to be produced [66].

Moreover, phthalic anhydride is used as protecting amino groups by heating the amino group with phthalic anhydride [67]. The study chitosan ester as chitosan phthalate was prepared by reacting chitosan with phthalic anhydride. The esteric form with a different solubility profile was insoluble in acidic condition and provided sustained release in basic condition, suggesting its suitability for colon-specific drug delivery systems [50]. In addition, shellac ester as shellac phthalate demonstrated to improve the thermal stability as compared to native shellac [68].

2.3 Degree of substitutions

Degree of substitution (DS): represents the number of the substitution groups which are in the molecular unit of the glucosamine units [69]. The DS is an important parameter when assessing the conversion of chitosan into one of its derivates, that influences the physicochemical properties such as solubility, chemical reactivity and biodegradability [9, 40, 53]. For example, the bioadhesive property of chitosan was enhanced by N-acylation with fatty acid chlorides. Chitosan modified with oleoyl chloride showed better mucoadhesion properties than chitosan modified with lower of %DS [70]. The release of drug is controlled by diffusion, or by swelling followed by diffusion, depending on both the acyl chain length and the degree of acylation. The resulted showed that palmitoyl chitosan has a substitution degree 40 – 50% the best mechanical characteristics and drug release properties [16]. The water-soluble chitosan-N-arginine with various DS from 8.7 to 28.4%, there were able to inhibit almost all the bacteria (*Staphylococcus aureus* and *Escherichia coli*) at a concentration higher than 150 ppm [55]. The preparation of amphiphilic derivatives of chitosan, propyl-succinyl-chitosan (HBP-SCCHS) by chemical modification. The DS of derivative was estimated by elemental analysis. The results showed that the increase of DS of propyl groups resulting in the surface tension decreases at the same concentration of the derivatives for there are more hydrophobic groups get to the surface of the solution (Figure 10) [28].

N-succinyl chitosan were prepared by reaction of chitosan with succinic anhydride at 1:1 w/w (SC1:1) and 1:3 w/w (SC1:3), respectively. DS of SC1:1 and SC1:3 were determined by FTIR and ninhydrin assays, presented the DS is 10 and 20%, respectively (Figure 11). The solubility of chitosan and its derivative were measured at three different pH. The result showed that chitosan was perfectly soluble in acid media but precipitated at neutral and alkaline solutions. The higher DS derivative (SC1:3) appeared insoluble in pH 4.0 due to the predominance of carboxylic groups compared to amino groups but completely soluble at high pH while the lower DS derivative (SC1:1) become partially soluble in the entire pH range due to the increasing substitution of the amino groups by carboxylic groups, which become negatively charged above pH 6.0 (Table 4). The result indicated that the ratio of reagent was factor affecting of DS [29].

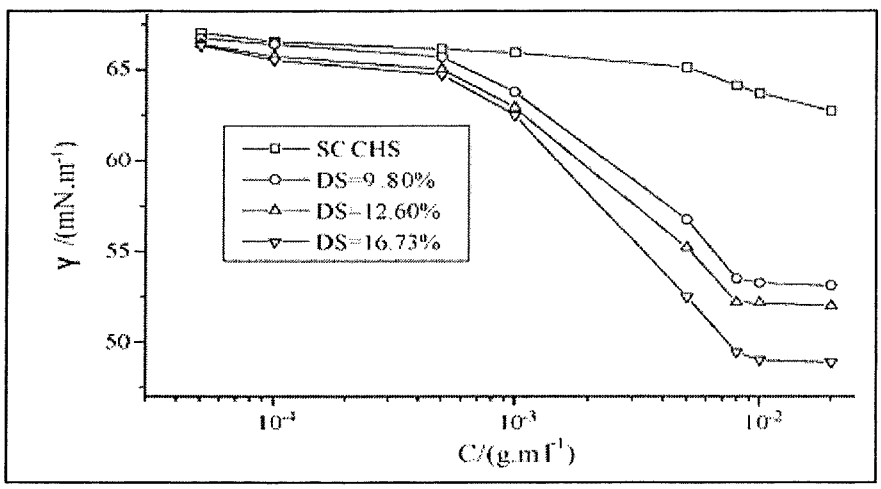

Figure 10 Surface tension–concentration plots of propyl-succinyl-chitosan (HBP-SCCHS) at different DS and N-succinyl chitosan (SC CHS).

Source: Sui, W. et al. (2008). "Preparation and properties of an amphiphilic derivative of succinyl-chitosan." **Colloids and Surfaces A: Physicochemical and Engineering Aspects** 316: 174.

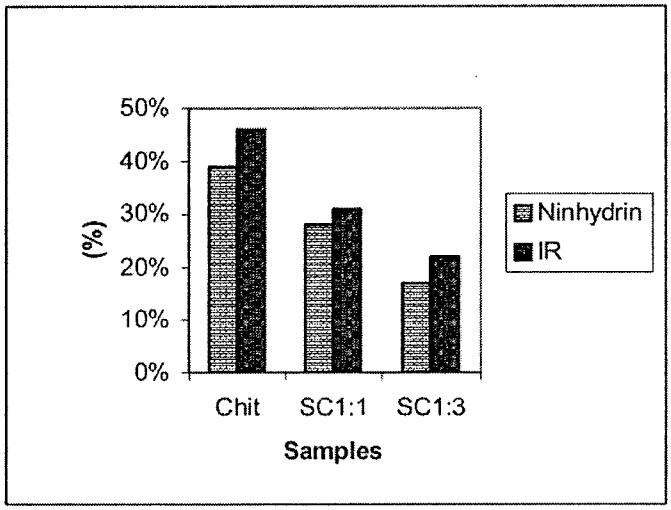

Figure 11 Degree of deacetylation of chitosan (Chit) and degree of substitution of N-succinyl chitosan prepared at 1:1 w/w (SC1:1) and 1:3 w/w (SC1:3) of chitosan and succinic anhydride determined by ninhydrin titration and infrared spectroscopy.

Source: Mello, K., et al. (2006). "Synthesis and physicochemical characterization of chemically modified chitosan by succinic anhydride." **Brazilian archives of biology and technology** 49(4): 667.

Table 4 Solubility tests of chitosan and N-succinyl chitosan prepared at 1:1 w/w (SC1:1) and 1:3 w/w (SC1:3) of chitosan and succinic anhydride in different solutions and pH.

Samples	**Solubility**		
	pH = 4.0	**pH = 7.0**	**pH = 10.0**
Chitosan	++++	-	-
SC1:1	++	++	+++
SC1:3	-	++	++++

(+, soluble; -, insoluble)

Source: Adapt from Mello, K., et al. (2006). "Synthesis and physicochemical characterization of chemically modified chitosan by succinic anhydride." **Brazilian archives of biology and technology** 49(4): 666.

The methods should be established to determine the exact DS, which can be used in manufacture, research and application of chitosan [71]. The chemical method, such as titration (ninhydrin assay) [72] or instrument methods, such as FTIR [16, 58, 73], NMR [24, 27, 40] and CHN elemental [55] analysis were used to determine the DS. However, all the methods have some limitation. In the case of chemical method, the operation is complicated and time-consuming. The instrument methods are normally expensive, but it is easy to be available, fast and can use solid sample directly [71]. The preparation of N-acylation of chitosan with various fatty acid chlorides for increasing hydrophobic character of derivatives [16]. The DS values were compared with ninhydrin and FTIR methods. The results showed no significant differences were noticed between the values obtained by these two assays (Table 5).

Table 5 Estimation of degree of substitution by ninhydrin and FTIR assays.

Samples	**Degree of substitution (%)**	
	Ninhydrin assay	**FTIR**
Nonmodified chitosan	15.4 ± 3.6	18.9 ± 2.1
Caproyl chitosan	43.6 ± 3.2	46.2 ± 4.2
Octanoyl chitosan	41.8 ± 3.3	43.9 ± 3.8
Myristoyl chitosan	45.6 ± 3.8	47.1 ± 2.7
Palmitoyl chitosan	44.4 ± 4.1	47.1 ± 3.6

Source: Adapt from Tien, C. L., et al. (2003). "N -acylated chitosan: hydrophobic matrices for controlled drug release." **Journal of Controlled Release** 93: 4.

2.4 Toxicity of chitosan and its derivatives

Chitosan is widely regarded as a nontoxic, biologically compatible polymer [74]. Chitosan has low oral toxicity with an LD_{50} in mice exceeds 16 g/kg [75]. Moreover, chitosan was compared to common sugars and concluded that chitosan is less toxic than these substances [76]. Toxicity of chitosan might depend on different factors such as degree of deacetylation, molecular weight, purity and route of administration [6]. Although, chitosan has been proved as a non-toxic polymer, but the modifications made to chitosan could make it more or less toxic and any residual reactants should be carefully removed before using it for biomaterials as drug delivery systems etc. The summary of toxicity of chitosan and its derivatives is shown in Table 6 [77].

For example, chitosan in the form of nano/microparticles was not affect the cell viability of the Caco-2 cells when using in the concentration 0.01-0.1 mg/mL [78]. Trimethyl chitosan has relative low cytotoxicity when it is an oligomer (3-6 kDa) with IC_{50}> 10 mg/mL for DS below 55%. Nevertheless, it was shown that trimethyl chitosan of increasing degree of trimethylation increased cytotoxicity as did higher molecular weight derivatives (100 kDa) [79]. In addition, *In vitro* cell toxicity testing of N-succinyl chitosan was not shown the effect on the activity of 3T3 fibroblasts when the concentration in the range of 0–0.25 mg/mL of N-succinyl chitosan nanospheres. These results demonstrate that N-succinyl chitosan is non-toxic, and cell-compatible. It can be safely used as the drug matrix (Figure 12) [26].

Table 6 Toxicity of chitosan and chitosan derivatives.

Chitosan details (DD, MW)	**Modification**	**Assessment**	**IC_{50}**
95% DD, 18.7 kDa	Steric acid conjugation micelle	*In vitro*, A549 cells	369 ± 27 μg/mL
95% DD, 18.7 kDa	Steric acid conjugation and entrapment in micelle	*In vitro*, A549 cells	234 ± 9 μg/mL
97% DD, 65 kDa	N-octyl-O-sulphate	*In vitro*, primary rat hepatocytes	> 200 mg/mL
87% DD, 20, 45, 200, 460 kDa	None, aspartic acid salt	*In vitro*, Caco-2 cells, pH 6.2	0.67 ± 0.24, 0.61 ± 0.10, 0.65 ± 0.20, 0.72 ± 0.16 mg/mL
87% DD, 20, 45, 200, 460 kDa	None, glutamic acid salt	*In vitro*, Caco-2 cells, pH 6.2	0.56 ± 0.10, 0.48 ± 0.07, 0.35 ± 0.06, 0.46 ± 0.06 mg/mL
87% DD, 20, 45, 200, 460 kDa	None, Lactic acid salt	*In vitro*, Caco-2 cells, pH 6.2	0.38 ± 0.13, 0.31 ± 0.06, 0.34 ± 0.04, 0.37 ± 0.08 mg/mL
87% DD, 20, 45, 200, 460 kDa	None, hydrochloride salt	*In vitro*, Caco-2 cells, pH 6.2	0.23 ± 0.13, 0.22 ± 0.06, 0.27 ± 0.08,0.23 ± 0.08 mg/mL
78% DD, < 50 kDa	None, lactic acid salt	*In vitro* B16F10 cells	2.50 mg/mL
82% DD, 150-170 kDa	None, lactic acid salt	*In vitro* B16F10 cells	2.00 ± 0.18 mg/mL
>80% DD, 60-90 kDa	None, glutamic acid salt	*In vitro* B16F10 cells	2.47 ± 0.14 mg/mL
77% DD, 180-230 kDa	None, lactic acid salt	*In vitro* B16F10 cells	1.73 ± 1.39 mg/mL
85% DD, 60-90 kDa	None, hydrochloric acid salt	*In vitro* B16F10 cells	2.24 ± 0.16 mg/mL
81% DD, 100-130 kDa	None, hydrochloric acid salt	*In vitro* B16F10 cells	0.21 ± 0.04 mg/mL
100% DD, 152 kDa	Glycol chitosan	*In vitro* B16F10 cells	2.47 ± 0.15 mg/mL
100% DD, 3-6 kDa	94% Trimethyl chitosan, chloride salt	*In vitro*, MCF7, 6 h	1.402 ± 0.210 mg/mL
100% DD, 100 kDa	36% Trimethyl chitosan, chloride salt	*In vitro*, COS7, 6 h	> 10 mg/mL
97% DD, 65 kDa	N-octyl-O-sulphate	*In vivo* , IV, mice	102.59 mg/kg
97% DD, 65 kDa	N-octyl-O-sulphate	*In vivo* , IP, mice	130.53 mg/kg

Source: Kean, T. and M. Thanou. (2010). "Biodegradation, biodistribution and toxicity of chitosan." **Advanced Drug Delivery Reviews** 62: 8.

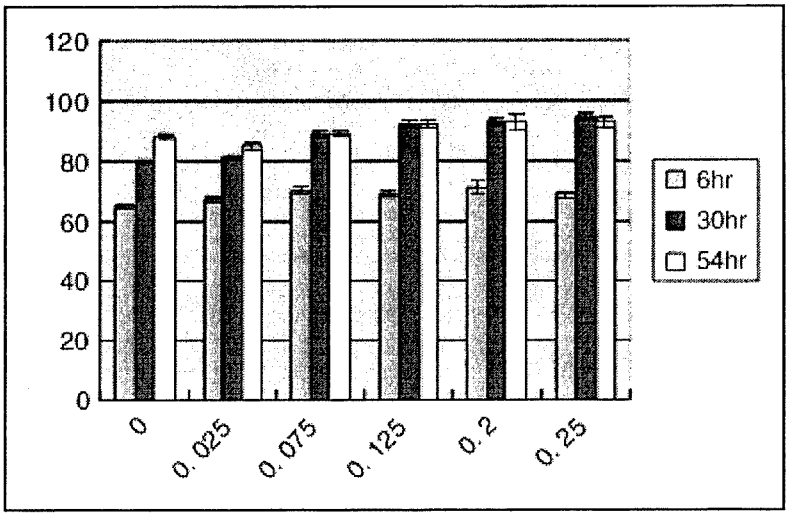

Figure 12 The dependence of the concentration of N-succinyl chitosan colloidal dispersion on the relative cell activity for a different period of cell culture.
Source: Aiping, Z., et al. (2006). "Synthesis and characterization of N-succinyl-chitosan and its self-assembly of nanospheres." **Carbohydrate Polymers** 66: 278.

2.5 Enteric coating

An enteric coating is a barrier applied to oral medication that controls the location in the digestive system where it is absorbed. Enteric refers to the small intestine, therefore enteric coatings prevent release of medication before it reaches the small intestine. Most enteric coatings work by presenting a surface that is stable at the highly acidic pH found in the stomach, but breaks down rapidly at a less acidic (relatively more basic) pH [80].

Purpose of enteric coating

Enteric coating is the most established of delayed-release products, designed to pass through the stomach unaltered, later to release their medication within the intestinal tract. There are three reasons for putting such a coating on a tablet or capsule ingredient:

1. To protect the stomach from the drug.
2. To protect the drug from the stomach, e.g. antibiotics, proteins, and peptides.
3. To release the drug after the stomach, e.g. in the intestines.

Enteric coating materials are also used to prevent release of the drug substance in the stomach if the drug is either an irritant to the gastric mucosa or unstable in gastric juice. List of enteric coating polymers commonly used in tablet formulations is shown in Table 7. The choice of enteric coating material depends on its solubility [81].

Table 7 Enteric coating polymers commonly used in tablet formulations.

Polymer	Solubility Profile	Comments
Shellac	Above pH 7	Original enteric coating material, originally used in sugar-coated tablets; high pH required for dissolution may delay drug release; natural product which exhibits batch-to-batch variability
Cellulose acetate phthalate (CAP)	Above pH 6	High pH required for dissolution a disadvantage; forms brittle films, so must be combined with other polymers
Polyvinylacetate phthalate (PVAP)	Above pH 5	–
Hydroxypropyl methylcellulose phthalate (HPMCP)	Above pH 4.5	Optimal dissolution profile for enteric coating
Polymers of methacrylic acid and its esters	Various grades available with dissolution occurring above pH 6	–

Source: Gad, S.C. (2008). **Pharmaceutical manufacturing handbook: production and processes**. North Carolina: John wiley & sons, Inc.: 894.