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# DRY CHITOSAN SALTS AND COMPLEXES OF ALIPHATIC CARBOXYLIC ACIDS

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## INTRODUCTION

Chitosan is the major outlet for chitinous products, largely because of its utility in the treatment of a broad range of potable and waste water systems for purification, clarification and solids concentration. Its safety and efficacy in reducing biological oxygen demand (BOD) are especially valuable. Currently, the practical use of chitosan requires the transport and storage of the formic or acetic acid solubilizing component and some expertise in control of solution preparation. These limitations might be avoided in many instances if dry, free-flowing carboxylic acid salts of chitosan were available. They could then simply be dissolved in water for their application.

Chitosan is soluble in a long list of aqueous acid systems<sup>1,2,3</sup>, but only a few dry chitosan salts have been described, such as the hydrochloride and sulfamate salts<sup>4,5,6</sup>. Cupery<sup>6</sup> indicates that unlike the sulfamate salt, addition of a solution of chitosan in aqueous acetic acid to methanol leads to a gel-like mass. Our experience with other organic acids and coagulants has been similar. In another approach, a water soluble chitin-chitosan of about 50% deacetylation has been prepared from alkali-chitin by low temperature hydrolysis<sup>7</sup>, but the adsorptive and other properties of this product may be different from normal chitosan of 80±% deacetylation.

We report here methods for the preparation of dry chitosan salts and complexes of the lower aliphatic carboxylic acids in a disperse system of chitosan, acid and an inert organic medium.

Heterogeneous reactions of polymers are notoriously difficult to control as reaction with the disperse polymer phase depends not only on particle size, but on its crystallinity, intra- and inter-molecular hydrogen bonding, rate of diffusion or penetration, and accessibility of reactive centers, in this case, the free amino groups of chitosan. This polymer itself is prepared in a heterogeneous system from chitin by reaction with hot 40-50% sodium hydroxide. Thus, chitosan is commonly deacetylated only to the extent of 75-90%, adequate even though non-uniform, to induce its solubility in 1-4% formic or acetic acid. However, with the strong attractive forces of the acid-base reaction, chitosan salt formation apparently proceeds effectively even in a heterogeneous system.

## MATERIALS AND METHODS

Low viscosity chitosan, Kytex from brown shrimp (*Penaeus aztecus*), was provided by Hercules Inc. (no longer suppliers); a low viscosity chitosan from Alaskan pink shrimp (*Pandalus borealis*) was obtained from the former Food Chemical and Research Laboratories (FCRL), now Protan, Redmond, Washington, and high viscosity crab shell chitosan, pharmaceutical grade, was supplied by Bioshell, Inc., Albany, Oregon. The materials varied in color; some had been bleached. All had specific gravities in the range of 1.44-1.45. Acids and reaction media were obtained from chemical supply firms and used as received.

In general, chitosan (0.5-25g, 8-40 mesh) was suspended in a selected organic medium, the appropriate acid added and the mixture allowed to stand for 1 to 3 hours. Temperature of reaction and drying varied between 12-35°C, but was usually at 20-25°C. For small sample reactions, occasional shaking appeared satisfactory; larger batches were stirred magnetically. The chitosan salts so formed were separated by filtration, washed with fresh medium, and the residual reaction media evaporated.

Viscosities were determined of a 1% aqueous chitosan salt solution with a Brookfield Synchro-Lectric Viscometer, Model LVF; measurements were made after solutions stood at ambient temperature for about two hours. Solution pH was determined with an Orion Research Digital Ionalyser 501. Specific gravities were approximated by the sink and float method.

Specifications indicated that the Kytex and Bioshell chitosans were deacetylated to the extent of 77-83%; hence, the figure of 80% was taken for unit equivalent weight calculations, i.e., a calculated unit molecular weight of 169. All of the chitosan samples were assumed to have this same value. Mole ratio of acid reacted or complexed per mole of chitosan was calculated from the gain in weight of the chitosan sample reacted.

## RESULTS AND DISCUSSION

Exploratory studies indicated that finely-divided chitosan can absorb a substantial amount of a low molecular weight carboxylic acid when the polymer is dispersed in organic media that are solvents for the carboxylic acids and non-solvents for chitosan. In general, most of the lower fatty acids up through four carbon atoms formed soluble chitosan salts<sup>3</sup>, which are dry, free-flowing solids. Reactions were somewhat specialized as to the media employed, but chlorohydrocarbons, ketones, esters, ethers, nitroaliphatics and acetonitrile were all applicable (Table 1).

Particle size of the chitosan appeared important in rate and extent of reaction; the finer sizes (20-40 mesh) are preferred. The acids vary in reactivity; formic and pyruvic acids appear most effective and their chitosan salts are the most stable. Temperature of reaction was more important than first thought (to be discussed later); ambient conditions were frequently satisfactory, but the 20-25°C range was consistently favored. Similarly, ambient temperatures were preferred for drying, as even at 40°C, some discoloration was encountered.

Initially, we chose reaction media on the basis of their solubility parameters, following the leads developed in solvent studies of the parent chitin<sup>8</sup>. However, it was found that several other factors may also be involved such as the dissociation and dielectric constants of the acids and media, complexities contributed by the presence of water and the molecular size of the acids.

Table 1. Chitosan Salts Formed in Various Media<sup>a</sup>

Acid	mL	Medium	mL	Yield g	Moles Acid Per Mole Chitosan	Sol. Rate <sup>b</sup>
Formic, 88%	1.5	Tetrahydrofuran	10	0.63	0.94	++
Formic, 95-97	1.0	Ethyl Acetate	10	0.65	1.09	+++
Formic, 95-97	1.0	Heptane	10	0.63	0.94	+
Formic, 88	NA	Acetone <sup>c,d</sup>	1	NA	-	+
Acetic (glacial)	1.0	1,2-Dichloroethane	5	0.69	1.06	++
Acetic (glacial)	1.0	Acetonitrile	5	0.65	0.83	++
Acetic (glacial)	1.0	Ethyl Acetate	5	0.57	0.39	±
Propionic	1.0	5% Water in Acetonitrile <sup>c</sup>	5	0.55	0.23	+
Isobutyric	2.0	Acetonitrile <sup>c</sup>	4	0.58	0.31	+
Chloroacetic	1.0	1,2-Dichloroethane	5	0.68	0.62	++
Dichloroacetic	1.0	1,2-Dichloroethane	5	0.75	0.63	+++

<sup>a</sup>In most cases, 0.5 g, 40 mesh, Kytex low viscosity chitosan was used with an excess of a molar equivalent of acid.

<sup>b</sup>Rate of solution: +++ = rapid; ± = viscous, not all soluble; pH of 1% solutions ranged from 5.3-5.8.

<sup>c</sup>High viscosity chitosan.

<sup>d</sup>Qualitative; NA = Not Available.

Another factor in this type of heterogeneous system is that of a distribution or partition coefficient of a third component that may be involved, that is, the distribution of a third component between two immiscible phases. In this case, the lower molecular weight carboxylic acid may distribute itself between the immiscible chitosan and the organic medium.

In a few cases, as with chitosan formate and acetate, the salt may be formed by reaction with an excess of the neat acid, i.e., without an inert medium, in which case the excess acid acts as its own medium. However, an additional inert reaction medium is usually preferred.

In these experiments, the physical recovery of a product is nearly quantitative. Yields lower than theoretical indicate that less than a stoichiometric amount of the acid reacted with chitosan and formed only a partial salt, or possibly that some of the salt dissolved in the medium. Such partial salts are water soluble, but in some cases may contain insoluble particles. Solubility of the partial salts may result from their heterogeneity. Perhaps they parallel the behavior of the heterogeneous but soluble 50% hydrolyzed chitin-chitosan of Sannan et al.<sup>7</sup> mentioned earlier.

Water content of the reaction system must be considered, as chitosan itself absorbs a substantial amount of moisture, particularly at higher humidities. In certain instances, it may be advantageous to add small amounts of water to act as a carrier or acid-dissociation medium and increase the reactivity of the system. The reaction of propionic acid and chitosan in acetonitrile containing 5% of water is a case in point (Table 1).

Table 2. Hydroxy Acid Salts of Chitosan

Acid		mL	Medium	mL	Yield g	Moles Acid Per Mole Chitosan	Sol. Rate <sup>a</sup>	pH
Glycolic,	70%	3	THF <sup>b</sup>	40	2.2 <sup>c</sup>	0.22	+++	5.3
Lactic,	85%	1	Ethyl Acetate	5	.73	.85	++	-
Lactic, ex.	85%	d	DCE	18	.76	.96	+++	6.4

<sup>a</sup>Rate of Solution: +++ = Most rapid.

<sup>b</sup>THF = Tetrahydrofuran; DCE = 1,2-Dichloroethane.

<sup>c</sup>From 2g high viscosity chitosan; low mole ratio may result from partial solubility of salt in reaction medium.

<sup>d</sup>1,2-Dichloroethane extraction from 85% lactic acid; reacted with 0.5g low viscosity chitosan.

Commercial formic, glycolic and lactic acids normally contain up to 30% of water for stabilization or avoidance of their decomposition on distillation. Such acids may require special media or techniques for best results. Thus, 95-97% formic acid may be used with 1,2-dichloroethane as the reaction medium, but with 70% glycolic acid or 85% lactic acid, tetrahydrofuran, ethyl acetate or other media that dissolve both water and acid are preferred for carrying out the reaction with the insoluble chitosan (Table 2). In some cases, the existence of azeotropes aids in the evaporative removal of water and media; the water dielectric may promote the reaction itself.

As an alternate procedure for acids containing significant amounts of water, e.g., 85% lactic acid, one may first extract the acid into the medium, remove an aqueous fraction and utilize the medium-acid portion for reaction with chitosan (Table 2). The chitosan lactate may be one of the most useful compositions because of its excellent solubility, stability and the higher pH of its aqueous solutions.

The pH of the different chitosan salt solutions varies with the acid component and perhaps the amount of acid absorbed by chitosan, as may be seen from Tables 1 and 2. However, they fall in the range of 5.3-6.4.

#### CHITOSAN SALT ACID SOLVATES OR COMPLEXES

The unusually slow drying of the chitosan formate and acetate led to the discovery that these salts were, in fact, carrying a surplus of the acid as a solvate or complex (Fig. 1). The residual reaction medium evaporated rapidly; slow but measurable release of the solvated acid then ensued. The solvated salts themselves are dry, free-flowing powders, relatively stable if kept cool in a closed container. Under ambient conditions the volatile acid is lost slowly, even beyond the simple stoichiometric chitosan salt composition. The chitosan formate is nearly odorless and remains soluble for up to a year, whereas the chitosan acetate continues to have a sharp odor and gradually loses acid until the salt becomes only partially soluble.

Examples of the preparation of the acid solvates are given in Table 3, including that of chitosan pyruvate. Significantly, only these smaller

Table 3. Acid Solvates or Complexes of Chitosan Salts

Chitosan Salt Solvate	Mole Ratio Acid to Chitosan		pH <sup>b</sup>	Vis., <sup>b</sup> cp
	Reaction <sup>a</sup>	Product		
Formate	4.5	2.47	4.2	8.3
Acetate	5.8	1.93	5.1	7.6
Pyruvate	4.8	2.67	3.5	5.1

<sup>a</sup>Kytex; low viscosity from brown shrimp; 1,2-dichloroethane reaction medium; 20-22°C for reaction and drying.

<sup>b</sup>One percent solution in water.

acid molecules appear to participate in solvate or complex formation involving more than a stoichiometric amount of acid.

The development of the chitosan salt acid solvates or complexes apparently is importantly a function of the molar excess of acid during salt formation. In the case of acetic acid (Table 4), a 5.6 molar ratio of acid to chitosan led to a substantial acid solvate, whereas a 2-3 molar ratio gave merely the simple soluble chitosan acetate partial salts. This series of chitosan acetates also showed a gradual decrease in specific gravity with increasing acetic acid content.

Pyruvic acid reacts rapidly with chitosan to yield both the normal salt and its acid solvate or complex, depending upon medium, the ratio of the reactants and temperature (Table 5). As a reaction medium, 1,2-dichloroethane was found most satisfactory. Since pyruvic acid is non-

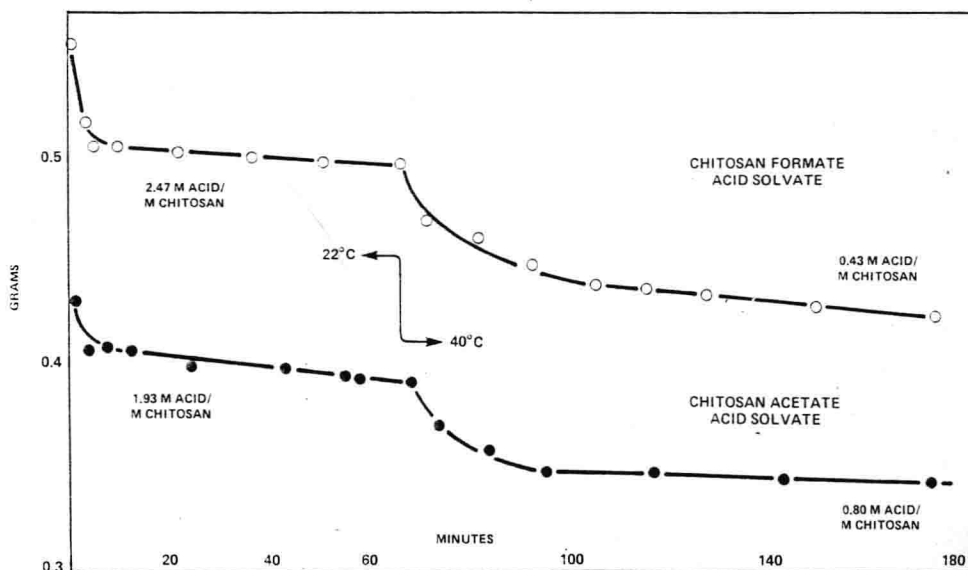


Fig. 1. Loss Rate of Acid Solvates of Chitosan Salts.

Table 4. Acid Ratios in Chitosan Acetate-Acid Solvate or Complex Preparation

Chitosan <sup>a</sup>		Acetic Acid		Moles Acid: Chitosan		Sol. <sup>b</sup> Rate <sup>b</sup>	Sp.Gr.
g	Moles	mL	Moles	React	Product		
5	0.03	10	0.17	5.6	1.56	+++	1.37
10	0.06	12	0.20	3.3	0.83	++	1.39-1.41
5	0.03	3	0.05	1.7	0.55	+	1.44

<sup>a</sup>FCRL pink shrimp; 1,2-dichloroethane medium; reacted at 14-20°C; 1-2.5h.  
<sup>b</sup>+++ = most rapid.

volatile, a range of compositions appears quite stable. The pyruvic acid reactions also illustrate the effect of raising the temperature over even a modest range in increasing the uptake of acid by chitosan. There appeared to be no regular effect on specific gravity of the salt by the amount of acid combined with chitosan.

A possible explanation for the formation of the acid-solvated or complexed chitosan salts, as well as perhaps some portion of the regular chitosan salts, may be that they fall into the class of intercalated biological systems<sup>10</sup>, that is, guest molecules inserted in a lamellar host. Several aspects of the preparation, physical properties and solution behavior of the solvates contribute to this postulate: (a) chitosan, like chitin, has a multiplanar structure<sup>11,12</sup>, a prerequisite for intercalation; (b) solvates or complexes are formed best with small, low molecular weight acids; the acid molecules, too, are flat; (c) the acid components of simpler salts as well as the complexes are present in variable ratios; (d) there is an apparent volume increase during the salt formation of certain solvates or complexes which is manifest in the lower specific gravity of the dry products; and (e) there may be a parallel in the behavior of chitin, which forms solvates with a number of low molecular weight alcohols and ketones<sup>13</sup>. Resolution of the conformation of the chitosan salts and their acid solvates or complexes, including their relation to the chirality of chitosan<sup>14</sup>, remains an intriguing problem.

## CONCLUSION

Dry chitosan salts or complexes can be obtained by the direct action of a lower aliphatic carboxylic acid on chitosan dispersed in an organic medium. These dry, free-flowing products dissolve readily in water and yield viscous solutions like the dissolution of chitosan itself in dilute aqueous acids. The chitosan salts or complexes of formic, pyruvic and lactic acid are favored for their ease of preparation, solubility and stability.

The heterogeneous reactions are carried out under ambient conditions in one to three hours; the product is filtered, washed with fresh medium and dried. Aliphatic monocarboxylic acids with up through four carbon atoms operate well. An excess of a molar equivalent of the acid is usually employed with an inert medium, such as chlorohydrocarbon, ester, ether, ketone or acetonitrile. Some water can be tolerated in the

Table 5. Effect of Temperature on Chitosan Pyruvate Preparation

React, <sup>a</sup> moles Acid : Chitosan	Temp. °C	Product, moles Acid : Chitosan	Sol. <sup>b</sup> Rate <sup>b</sup>	Sp.Gr.
1 : 1	30-35	0.31	-	1.45
3 : 1	30-35	1.59	+++	1.44-1.45
5 : 1	30-35	2.32	+++	1.45-1.46
4.7 : 1	18	1.21	+++	1.42-1.44
3 : 1	13	0.57	±	1.44
5 : 1	13	0.88	++	1.42-1.44

<sup>a</sup>0.34g Kytex low viscosity chitosan, Sp.Gr., 1.45; 5 mL 1,2-dichloroethane.

<sup>b</sup>+++ = most rapid, ± = viscous solution but not all soluble.

reaction, as both 88% formic acid and 70% glycolic acid can be used in an appropriate medium.

A novel feature of this method is the development of chitosan salt acid solvates or complexes with more than a molar equivalent of acid when a substantial excess of the acid component is employed in the reaction. It is possible that these solvates or complexes may be intercalation products. Chitosan complexes with formic and acetic acids lose acid slowly unless confined, but complexes of less volatile acids such as pyruvic acid are quite stable.

These solid chitosan salts or complexes, soluble in water, offer advantages of convenience, ease of control and simplicity in handling. The complexes with more than a molar equivalent of acid provide a reservoir of acid for good solubility, for adjusting pH and for solubilizing basic components in certain formulations.

#### ACKNOWLEDGMENT

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# CHEMICAL MODIFICATIONS OF CHITIN AND CHITOSAN

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## INTRODUCTION

Although chitin may be less advanced than cellulose with regard to research and utilization, it has recently begun to attract much attention in both basic and applied research fields, including not only biology and biochemistry but also organic and polymer chemistry, pharmacology, and medicine. The number of reports and patents is increasing at a remarkable rate. Besides basic research, many attempts have been made to find new applications for chitin. Further basic studies, however, seem to be necessary to realize its full potential. One possible breakthrough in chitin chemistry and technology is the development of chemical modifications of chitin which are being studied more and more actively to explore highly sophisticated functions. Chitin can be regarded as a new type of polymeric material having greater possibilities than cellulose in many respects. Since it is an amino polysaccharide, chitin is capable of undergoing many additional modification reactions. It is anticipated that widespread applications will be found in the near future.

This review deals with the chemical modification of chitin and chitosan, focusing on the recent developments, to see the possibility of exploring highly useful functions in perspective. The following modification reactions will be discussed along with the resulting properties: deacetylation, acylation, aldimination, carboxymethylation, sulfation, complexation with metal cations, and some miscellaneous reactions.

### Deacetylation

On treatment of chitin with base, the acetamido groups are hydrolyzed to give free amino groups. This hydrolysis to chitosan is usually carried out with sodium hydroxide or potassium hydroxide at elevated temperatures under heterogeneous conditions using chitin flakes or powders. In spite of the alkaline hydrolysis, the polysaccharide main chain is also degraded to some extent. The reduction of molecular weight becomes pronounced with increasing reaction time (1). An attempt was recently made to reduce the extent of depolymerization as much as possible in preparing fully deacetylated chitosan (2). The deacetylation was carried out similarly but with added thiophenol which traps oxygen thus preventing degradation and exerting a catalytic effect.

For instance commercial chitosan with 80 % deacetylation becomes fully deacetylated by repeating three times the treatment with sodium hydroxide in water/DMSO in the presence of thiophenol at 100° C for 1 h. The mole-

cular weight decreases with increasing number of treatments, but the reduction is less extensive than with conventional treatment.

The hydrolysis can be conducted under homogeneous conditions as well as the conventional heterogeneous conditions. The conventional hydrolysis at gentle boiling depends on the alkali concentration, and at low concentrations, the degree of deacetylation levels off at relatively low values. With 40 % sodium hydroxide, deacetylation proceeds to a high extent. The samples with 45 to 55 % deacetylation were found to be water-soluble, whereas those with above 60 % or below 40 % deacetylation are naturally insoluble in water (3-5). This remarkable water-solubility is probably brought about by random deacetylation along the chains, since the sample with 50 % deacetylation prepared by heterogeneous deacetylation is not water-soluble.

### N-Acylation

The N-acetylation of chitosan leads to fully N-acetylated chitin. In studying the influence of the reaction medium on the N-acetylation of chitosan, it was found (6) that maximum reaction rate is achieved using binary mixtures of ethanol and methanol or methanol and formamide. Complete N-acetylation may be achieved in three minutes at room temperature using a highly swollen chitosan in organic aprotic solvents (7). Alternatively, the preparation of partially acylated chitosans was made by treating solutions of chitosan in acetic acid-methanol with acid anhydrides at room temperature. Rapid N-acylation of chitosan can be achieved, in fact, under heterogeneous conditions if highly swollen gels are prepared or the sample has been steeped in solvent (7-9). Homogeneous acylation is also possible in trichloroacetic acid/dichloroethane (10). Although methanesulfonic acid is a strong acid, it is used as a solvent (7). Chitin is insoluble in the solvent, but the acylated products go into solution. The structure of the acyl residue has considerable influence on the ease of O-acetylation, and with bulky acyl groups, steric hindrance may prevent substitution beyond 0.5. Under these conditions, the formation of highly acetylated products requires prolonged reaction times.

Long-chain di-O-acyl chitins may be prepared upon reaction with acyl chlorides or anhydrides in methanesulfonic acid at low temperature (11). Chitosans boiled with a large excess of hexanoyl, decanoyl or dodecanoyl chlorides in dry pyridine-chloroform afford fully acylated derivatives (12). Highly benzoylated chitin shows good solubility in several organic solvents (13). An aspirin carrier was prepared by reaction of chitosan with 2-acetoxybenzoic anhydride (14).

Carboxyacyl chitosans have been prepared from reactions with anhydrides of alkyl and aryl dicarboxylic acids (15-17). The acylation with cyclic anhydrides such as trimellitic anhydride and pyromellitic anhydride on water-soluble chitin gives amic acid-chitins, which are transformed into imide-chitins. These derivatives have newly introduced carboxyl and acid anhydride groups, respectively, available for further modification (15).

Partially succinylated chitosans and O-hydroxyethyl chitosan containing both amino and carboxyl groups show varying solubility in water, dilute acid and dilute alkali, depending on the degree of substitution (16). By further reaction with carbodiimides, gels with a low degree of cross-linking have been prepared (16-18). Acetylated chitosan is obtained as porous particles when an aqueous chitosan solution is treated with a toluene solution of acetic anhydride (19). These particles are useful as molecular sieves, and dextrans with different molecular weights were demonstrated to be effectively separated. Wetting characteristics of acylated chitin film surfaces and blood clotting on the surfaces have been studied to evaluate these derivatives as biocompatible materials (20). Table I summarizes some examples of blood clotting

time. In this series, 2.0 acetylchitin shows the best result. All acylated chitins behave similarly to siliconized glass.

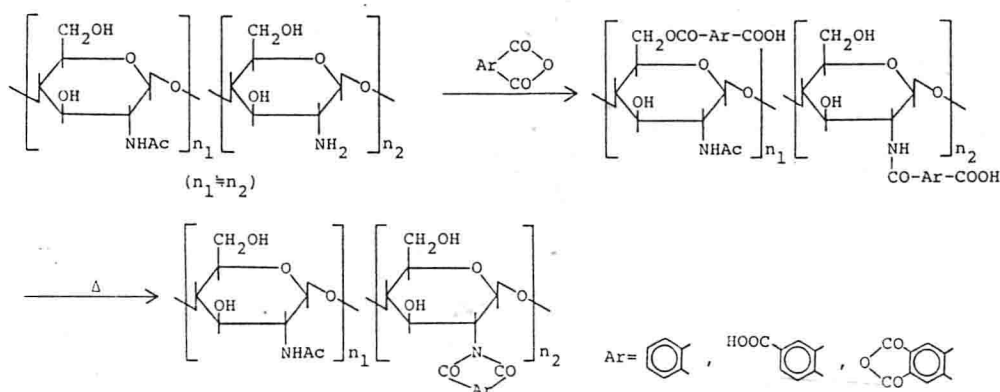


Table I Relative clotting time index

Sample	Clotting time ratio	
	Sample/Glass	Sample/Siliconized glass
Chitin	1.3	0.7
1.4 Formylchitin	1.6	1.0
2.0 Acetylchitin	3.8	1.9
1.9 Propionylchitin	2.4	0.9
1.8 Butyrylchitin	2.0	1.2
2.0 Caproylchitin	2.5	1.1

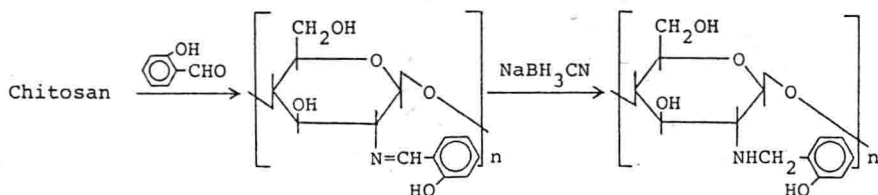
Some of these derivatives show solubilities analogous to proteins, in being soluble in dilute sodium hydroxide, precipitating at pH 4 - 5 and dissolving at low pH values.

Although the maleylation of amino groups in proteins may be carried out in water at pH 7.1 - 9.5, the heterogeneous reaction with chitosan was better performed in formamide (21). The copolymers of N-maleylchitosan and acrylamide are stable at all pH values; they swell in water and give gels with good mechanical properties. Immobilized antibodies on these materials are effective in reducing hepatitis virus antigen values in blood plasma (22).

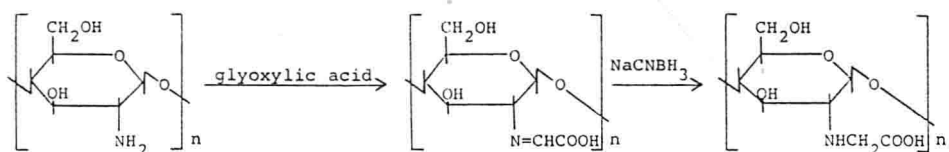
#### Schiff bases and their reduction products

The Schiff reaction between chitosan and aldehydes or ketones gives the corresponding aldimines and ketimines, which can be hydrogenated to products less susceptible to hydrolysis (23). N-Alkyl chitosans from simple aldehydes and ketones have been isolated as white powders with degrees of substitution 23 - 33 % (24). Intramolecular hydrogen bonds are apparently weakened by the presence of the bulky substituents and thus, despite their hydrophobicity, the N-alkyl chitosans swell enormously in water; they retain the film-forming ability of chitosan and membranes can be cast from their acetic acid solutions.

The reductive amination reaction of chitosan with polyfunctional aldehydes and ketones provides access to polyampholytes, such as the one obtained from salicylaldehyde.



The addition of phthalaldehydic acid or glyoxylic acid to aqueous suspensions of chitosan resulted in immediate dissolution, with accompanying gel formation at suitable pH values (25-27). The Schiff bases, upon hydrogenation, afforded N-(o-carboxybenzyl) chitosan (NCBC) and N-(carboxymethyl) chitosan (NCMC), soluble in both acidic and alkaline media (see Figure 1). In this compound, each carboxymethylamino residue is simultaneously a secondary amine and a carboxylic acid.



The n.m.r. spectrum for a typical NCMC shows signals attributable to the N-carboxymethyl substituent, at 168.7 and 47.7 ppm, for  $\text{COO}^-$  and  $\text{N}-\text{CH}_2$ , respectively. A downfield shift ( $\sim 6$  ppm) is observed for the carbon bearing the N-carboxymethyl group, C-2', with respect to the corresponding

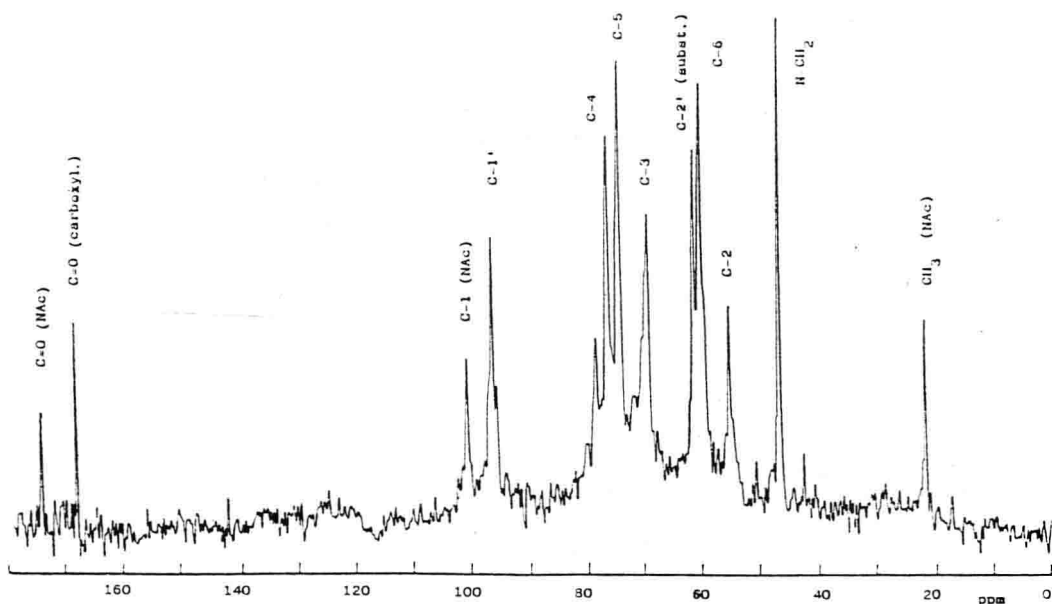


Fig. 1.  $^{13}\text{C}$ -n.m.r. spectrum of a typical N-carboxymethyl chitosan with degree of acetylation 40 %, degree of N-carboxymethylation 50 % and free amine 10 %. Courtesy of R.A.A. Muzzarelli (29).

carbon of unmodified residues, C-2 at 57.8 ppm. From the ratio of the areas of signals C-2 and C-2' (as well as the corresponding anomeric carbons C-1 and C-1'), N-acetylation is about 40 % and N-carboxymethylation more than 50 %.

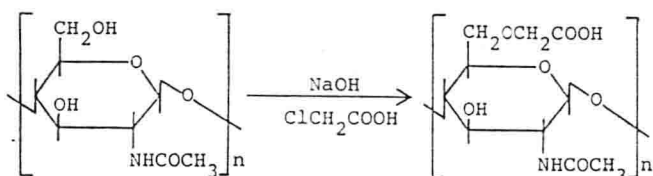
Some similarities exist between O-carboxymethyl chitosan and N-carboxymethyl chitosan in physical properties (28) (moisture content, fall in viscosity during the first few days after preparation, coagulation by solvents and neutral salts), but the primary alcohol groups of NCMC are available for further reactions such as cross-linking (29).

The attachment of reducing carbohydrates as side-chains to the 2-amino functions of chitosan transforms it into branched-chain water-soluble derivatives. Easy conversion can be achieved by reductive alkylation using sodium cyanoborohydride (23, 30). Further, specific chemical modifications can be effected with the introduction, via D-galactose oxidase treatment, of aldehyde functions into the pendant D-galactose residue. Reductive alkylation of chitosan with lactose affords 1-deoxylactit-1-yl chitosan whose solution has unusual rheological properties.

### O-Carboxymethylation

Carboxymethylation is achieved with monochloroacetic acid and sodium hydroxide (31). The reaction takes place preferentially at C-6 hydroxyl groups (32). In this reaction, however, some part, sometimes over 50 %, of acetamido groups are inevitably hydrolyzed on account of the strongly basic conditions. In a modified method, DMSO is used to swell chitin (33).

Crosslinked carboxymethylchitins or chitosan show high capability of separating bovine serum fibrinogen and albumin. The adsorption behavior of bovine blood proteins on carboxymethylchitins with various substitution degrees was also studied by Tokura and coworkers (34).



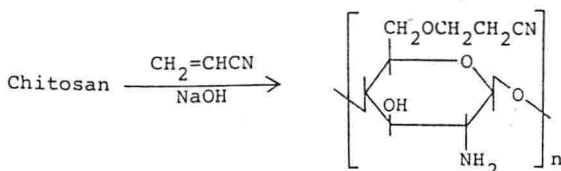
An interesting application of carboxymethylchitin was reported recently in the preparation of artificial red blood cells (35). A W/O type emulsion obtained from sheep hemolysate and lecithin in dichloromethane is dispersed in an aqueous carboxymethylchitin solution to form a W/O/W type emulsion. Removal of the organic solvent results in an aqueous suspension of artificial red blood cells.

### Sulfation

Sulfation of chitin and chitosan has been one of the most attractive modification fields owing to the possibility of preparing anticoagulant polysaccharides in view of the structural similarity to heparin. For sulfation, various reagents have been used including concentrated sulfuric acid, oleum, sulfur trioxide/pyridine, sulfur trioxide/sulfur dioxide, and chlorosulfonic acid, but the last one is most commonly used. The present Volume includes Chapters devoted to the preparation and uses of sulfated chitosans.

### Miscellaneous Reactions

Cyanoethylation with acrylonitrile is carried out in a manner similar to



the preparation of cyanoethylcellulose. Cyanoethylchitosan, on blending with cellulose nitrate, gives membranes which are good for microfiltration (36). An advantage of these membranes is that almost no shrinkage occurs on autoclave sterilization.

Graft polymerization onto chitin is another interesting way to modify its properties, but only a few reports have been published. The grafting of methyl methacrylate onto chitin takes place with tributylborane as the initiator by a radical mechanism (37). Styrene is also grafted with  $\gamma$ -ray irradiation (38). In both cases, water plays an important role in promoting the graft polymerization. Further reactions are described in the following Chapters.

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