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Chitin and Its Derivatives: New Trends of Applied Research

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SUMMARY

The applications of chitin, chitosan and a number of their chemical and natural derivatives, proposed in very recent publications and patents (1977–80) are reviewed in various fields, including fibre and film manufacture, as paper additives, in metal ion recovery, as semi-synthetic polymers, flocculants, fungicides and a number of aspects of biochemistry. A major trend in chitin applied research is towards more sophisticated applications dealing with immunochemistry, medical aids, enzyme immobilisation and membrane technology. Chitin, therefore, represents an important renewable resource.

INTRODUCTION

Chitin, [(1,4)-2-acetamido-2-deoxy-β-D-glucan], the most widespread polysaccharide in living organisms (Muzzarelli, 1977), is currently the subject of a number of applied research projects, directed towards the commercial exploitation of various chitinous resources. ANIC, Milano, has installed pilot plants in Italy and Norway to produce chitin and chitosan from shrimp and from *Aspergillus niger* (Muzzarelli, 1979; Nicolaysen, 1980; Muzzarelli *et al.*, 1981a, b); other European sources are krill processing wastes (Maciejczyk, 1980). In the USA, chitin and chitosan are currently produced from shrimps and crabs from Alaska and Mexico (Hattis & Murray, 1976). In Japan, about two thirds of the

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local resources are already used in several applications (Muzzarelli, 1977; Tokura & Nishi, 1977; Takemoto, 1981).

During the First International Conference on Chitin and Chitosan, held in Boston in 1977 (Muzzarelli & Pariser, 1978), considerable interest was shown in the industrial applications of chitin, especially in the textile and paper industries and in medicine. Applications considered included the recovery of metal ions, the immobilisation of enzymes and flocculation. Moreover, it was shown that chitosan was a reactive polymer susceptible to many chemical modifications (Muzzarelli, 1978a). Several chitin derivatives were described at that time (Muzzarelli, 1978b)and further derivatives have been produced very recently. These are listed in Table 1.

TABLE 1
Chitosan Derivatives and Proposed Uses

N-Acyl chitosans: acetyl, propionyl, butyryl, hexanoyl, octanoyl, decanoyl, dodecanoyl, tetradecanoyl, lauroyl, myristoyl, palmitoyl, stearoyl, benzoyl, dichloroacetyl, carbamoyl (textiles, membranes).

N-Carboxyalkyl chitosans: N-carboxymethyl (metal ion collection).

N-Carboxyacyl chitosans: from anhydrides such as: maleic, itaconic, (acetylthio)-succinic, glutaric, cyclohexane-1,2-dicarboxylic, phthalic, *cis*-tetrahydrophthalic, 5-norbornene-2,3-dicarboxylic, diphenic, salicyl.

O-Carboxyalkyl chitosans: O-carboxymethyl, epichlorohydrin-cross-linked O-carboxymethyl (membranes, molecular sieves).

Deoxyglycit-1-yl chitosans: 1-deoxygalactit-1-yl, 1-deoxyglucit-1-yl, 1-deoxymelibiit-1-yl, 1-lactit-1-yl (gels, drilling muds).

Metal ions – chitosan chelates: palladium, copper, silver, iodine (catalysis, photography, health products, insecticides).

Semi-synthetic resins of chitosan: methyl methacrylate, poly(urea-urethane) poly(amide-ester), acrylamide-maleic anhydride-chitosan copolymer).

Natural polysaccharide complex: chitosan-glucan (flocculation, metal ion chelation).

Miscellaneous: alkali chitin (intermediate), benzyl chitin (serine protease purification), hydroxybutyl chitosan (desalting), cyanoethyl chitosan (filtration, dialysis, insulating papers), glycol chitosan (dialysis, special papers), glutaraldehyde chitosan (enzyme immobilisation), linoleic acid–chitosan complex (food additive, anticholesterolaemic), uracyl chitosan, theophylline chitosan, adenine chitosan, chitosan salts of acidic polysaccharides.

TEXTILES AND FILMS

Fibres

The poor solubility of chitin in most solvents is due to its micellar structure caused by hydrogen bonds involving the acetamido groups. Chitin can be dissolved, however, in a number of new ways: for instance, a chitin suspension in formic acid, frozen at −20°C for 24 h, yields a turbid gel which becomes clear upon addition of a small amount of trichloroacetic acid. The resulting chitin solution, with isopropyl ether added to lower its viscosity, was spun through a nozzle into an ethyl acetate coagulating bath. The resulting fibres were fully characterised and their potential usefulness in the textile industry was emphasised by Tokura *et al.* (1979). The use of methanesulphonic acid was also proposed by Noguchi *et al.* (1978a, b, c). Fibres and films made of acylated chitin were also made by treating chitin with acetic anhydride in trichloroacetic acid and 1,2-dichloroethane mixtures (Ando & Kataoka, 1980). A variety of similar mixtures also allowed the preparations of fibres and films (Agency of Industrial Sciences & Technology, 1980a).

A water-soluble alkali chitin can be prepared by the combined actions of steam heating under pressure, freezing, pressure at 50 kg cm^{-2} and treatment with concentrated NaOH (Mitsubishi Rayon Co., 1980a, b, c). This material is not only suitable for the manufacture of films and fibres, but also useful for further derivatisation (Noguchi, 1978).

N,N-Dimethylacetamide and LiCl mixtures are also good solvents for chitin (Rutherford & Austin, 1978a): from such solutions, carbamates and esters were prepared for use in the insecticide field (McCormick *et al.*, 1980). A new type of fire retardant polymer was obtained from chitosan and hexachlorocyclotriphosphazene (Allan *et al.*, 1981).

Membranes

Membranes can be manufactured from solutions of chitin and its derivatives. Chitin membranes have been tested as semipermeable membranes with 0·1% dextran (mean M.W. 75 000) and found useful for dialysis by Ando & Kataoka (1979a, b). The rejection efficiency of acetyl chitin toward amino acids, sugars, salts and polyethyleneglycol was 7500, compared to 10 000 for chitin (Cho & Kong, 1980). Membranes

obtained from chitosan and polyethyleneglycol showed an elongation of 110%, a water permeation of 0·20 ml min^{-1} and a vitamin B_{12} permeability of $1{\cdot}9 \times 10^6$ cm^2 s^{-1}, compared to 140, 0·0045 and 0·38 respectively for chitosan membranes (Agency of Industrial Sciences & Technology, 1980b).

Chitosan, N-acyl chitosan and N-arylidene chitosan membranes have been studied in terms of their stability in various solvents. The water permeation rate for N-acetyl chitosan (thickness 12–60 μm) was 0·144–0·340 kg m^{-2} day^{-1} under an applied pressure of 3 kg cm^{-2}, while it was 0·010 kg m^{-2} day^{-1} for 30–35 μm thick chitosan membranes. These membranes were permeable to various sugars with M.W. up to 2900 and impermeable to cytochrome c of M.W. 13 000 (Hirano *et al.*, 1980).

Hydroxybutyl chitosan in dimethyl formamide was used to prepare 1·3 μm thick membranes for brine desalination: a 0·2% brine was passed through the membrane under an applied pressure of 40 kg cm^{-2}, the water permeation rate was 20 kg m^{-2} day^{-1} and the salt concentration was reduced by 72·1% (Matsuda *et al.*, 1976). A new membrane for microfiltration was produced from a mixture of cellulose nitrate and cyanoethyl chitosan, and used to prepare ultra-pure water and pharmaceuticals by Kesting (1979). Glycol chitosan–mucopolysaccharide complexes have been found suitable for the separations of urea, glucose and sucrose by Nakajima & Shinoda (1977). The reaction of chitosan with sodium isocyanate gave N-carbamoyl chitosan suitable for the preparation of fibres and disposable films, insoluble in water, acids, alkali and organic solvents (Daini Seikosha Co., 1980; Dainichisecka Color & Chemical Mfg. Co., 1980a).

Mixtures of trichloroacetic acid (40%), chloral hydrate (40%) and methylene chloride (20%) dissolve chitin (3% w/w in 30 min): from such solutions threads and membranes suitable for surgical sutures and food wrapping can be obtained (Austin & Brine, 1977). Chitosan reacts easily with mono-, di- and trichloroacetic acids under mild conditions to form corresponding chitosan derivatives which are soluble in acetone. The films obtained from these chloroacetylated chitosans are converted into hard and insoluble films after crosslinking by irradiation with UV light (Tanaka *et al.*, 1980). Crosslinking is accelerated by naphthalene (Japan Synthetic Rubber Co., 1981).

Copper–chitosan films find uses in photography: they are interposed between the silver precipitating layer and the silver halide emulsion layer. The copper–chitosan chelate improves the photosensitivity

because copper catalyses the reduction of complexed silver; such an application is possible because of the chitosan film permeability to silver complexes (Scott, 1977). Chitosan membranes are selectively permeated by certain ions and complexes: the transition metal cations are chelated by chitosan and, as a consequence, they slow down the permeation by similar cations without altering the permeation by alkali metal ions (Muzzarelli *et al.*, 1980c; Blair & Ho, 1981). Other proposed applications were for food packaging, recording and video tapes (Kataoka & Ando, 1978a, b, 1979a). Chitin–poly(5-methyl L-glutamate) blend films possess good mechanical strength (Kataoka & Ando, 1979b).

PULP AND PAPER

Hydrogen bonds exist between the separate segments of crossing fibres which together make up the interfibre bonding area: water has a disruptive effect on the cohesiveness of paper, because the interfibre linkages are broken and hydrogen bonding sites on the fibre surfaces are occupied by water molecules.

Chitosan obviates the effects of water; it has been added to various types of paper (letter, newsprint, map, cardboard and cigarette paper) with satisfactory results (American Cyanamid Co., 1978; Allan *et al.*, 1978b; Kashiwabara, 1978). Fungal chitosans, such as chitosan–glucan complexes from *Aspergillus niger* and *Mucor rouxii* are also good paper additives (Muzzarelli, 1978b; Muzzarelli, 1979; White *et al.*, 1979; Muzzarelli *et al.*, 1982). Several species were also examined by Johnson & Carlson (1978) for their ability to grow on spent liquors from the pulp and paper industry.

Complexes of cellulose and chitosan were tested for sorption of dyes from waste water with results better than with commercial activated carbon (Shinagawa *et al.*, 1979), because the sorption capacity of chitosan was higher. Kimura (1979) partially oxidised cellulose to aldehyde and condensed this with chitosan, to obtain a polymeric Schiff base superior to activated charcoal in the treatment of water containing I.C. Acid Blue 40. According to Belen'kaya *et al.* (1979), the surface treatment of various types of paper with a 1% chitosan solution increases the breaking length, bursting strength and folding endurance of the paper, with no effect on its brightness. There are a number of possible applications for this, including the restoration of antique manuscripts. Such treatment reduces the consumption of rosin for

sizing and enhances the printing properties, particularly in the manufacture of maps and punched cards (Baranova & Plisko, 1980).

Cyanoethyl chitosan at a usage level of 0·3–0·5% of cellulose was recommended by Baranova *et al.* (1976) for the production of electrical insulating papers: the electrical resistance of the paper can also be increased by using chitosan (Flyate *et al.*, 1980). The degree of retention of starch by paper is increased by the addition of 0·06–0·16% chitosan (Baranova *et al.*, 1980). In this respect glycol chitosan was compared to six other cationic polymers and found to give the best performance by Tanaka *et al.* (1979).

METAL ION RECOVERY

Chitosan exhibits chelating ability toward a number of metal ions, because its hydroxy and amino groups act as electron donors (Muzzarelli, 1977). The chelating abilities of chitin and chitosan have been confirmed with the use of advanced instrumental techniques such as EDAX (Energy Dispersive Analysis of X-rays) and ESCA (Electron Spectroscopy for Chemical Analysis) by Eiden *et al.* (1980), as well as by studying the metal ion complexes of D-glucosamine (Miyazaki & Nishimura, 1979).

Industrial solutions containing Ni (7 ppm) in the presence of excess Na (10 g litre^{-1}) have been passed through chitosan columns to remove Ni selectively (Randall *et al.*, 1979). Plutonium bearing waters at various pH values in the range 4·94–10·24 have been treated with chitin and decontaminated (83% reduction in plutonium at pH 4·94); Pu was then recovered after ashing (Silver, 1978). Sulphur dioxide treated chitosan (6 g) was used to remove chromate (1000 ppm) from waters (2·2 litre) used to colour wool (Masri & Randall, 1978). In addition to glutaraldehyde, some monosaccharides and salicylaldehyde were used to enhance the performances of chitosan by taking advantage of the complexing ability of the Schiff bases (Hall & Yalpani, 1980).

The chelating ability of the chitosan–glucan complex obtained from the mycelia of *Aspergillus niger*, was assessed by Muzzarelli (1978a, b, 1979); this complex, which was fully characterised, has a higher chelating capacity than chitosan from crustacea; the waste source material is a well-characterised raw material which derives from established industrial fermentation processes (citric acid production) (Muzzarelli *et al.*, 1980a, b, c).

The chelating ability of chitosan is not only useful for the recovery of metal ions but also for supporting catalysts. Polyenes and alkynes were treated with hydrogen in the presence of Pd catalysts supported by chitosan and amines, to give mono-olefins selectively (Noguchi Institute, 1981). The initial step was the fixation of potassium tetrachloropalladate on chitosan, in the same way as previously disclosed by Muzzarelli (1972). Similarly, Arena (1981) collected metal ions using the method described by Muzzarelli (1972) and then reduced the metal ions with hydrogen or sugars to yield a dispersed metal catalyst for hydrogenation, isomerisation and oxidation.

Chitosan was cross-linked with glutaraldehyde to render it insoluble in the acidic media from which metal ions had to be removed: chitosan was also found suitable for the removal of BF_4^-, ZrF_6^{2-}, TiF_6^{2-}, SbF_6^-, at pH 4–5 (Masri *et al.*, 1978).

Non-metals

The adsorption of bromine on chitin and chitosan takes place in aqueous mixtures of bromine and potassium bromide at 30°C (the chitosan membrane adsorbs 0·689 g Br g^{-1} chitosan after 26 h at 20°C, or 0·812 g from alcoholic solutions under the same conditions). When chitosan adsorbs iodine, swelling occurs due to structural alterations. The adsorption of iodine is caused by a charge-transfer mechanism between iodine and amino groups in chitosan, similar to those involving nylon-6 and poly(vinyl anthracene) but different from those involving amylose and poly(vinyl alcohol). The chitosan membrane adsorbs 1 g/g of chitosan from 0·01 M iodine in ethanol, and the complex is stable up to 190°C. Sodium thiosulphate removes iodine and yields opaque membranes or amorphous powders (Kato *et al.*, 1979; Shigeno *et al.*, 1980a, b, 1981).

SEMI-SYNTHETIC POLYMERS

Copolymers of glucosamine

Semi-synthetic polymers containing saccharide units are of interest because they can combine the advantages of natural and synthetic polymers and can be tailored to meet certain industrial needs.

Chitin reacts with methyl methacrylate in the presence of tributylborane and water at room temperature, but it does not react in organic solvents. Grafting proceeds via a free-radical mechanism after solvation of water to chitin and the formation of the complex from solvated chitin and tributylborane (Kojima *et al.*, 1979).

Poly(urea-urethane)s containing D-glucosamine units in the main chain were prepared by Kurita *et al.* (1979a, b) by direct addition polymerisation of D-glucosamine and di-isocyanates, in N,N-dimethylacetamide in the presence of triethylamine and without any protection of the secondary alcoholic functions. The products are soluble in polar solvents such as N,N-dimethylacetamide, N-methyl-2-pyrrolidone, dimethyl sulphoxide, hexamethyl phosphoric triamide and dichloroacetic acid, but insoluble in acetone, methanol, benzene and chloroform.

The synthesis of poly(amide-ester)s was similarly carried out by direct polycondensation of D-glucosamine and dicarboxylic acid chlorides such as terephthaloyl, isophthaloyl and adipoyl chlorides: white powders are obtained whose solubilities are similar to those of poly(urea-urethane)s with good thermal and oxidative stabilities, and high degrees of crystallinity, especially for the polymer derived from adipic acid (Kurita *et al.*, 1980).

Optically active polymers

In the presence of chitosan, the radical polymerization of sorbic acid is initiated by potassium persulphate in water at 50°C and leads to poly(sorbic acid) which has a 1,4-*trans* type structure and shows optical activity (Kataoka & Ando, 1980a). Similarly, polymethacrylic acid and its methyl ester obtained in the presence of chitosan shows optical activity opposite in sign to that of the chitosan used as the matrix; this optical activity is retained after removal of chitosan by chemical hydrolysis. The isotactic content of these polymers is larger than that obtained in the absence of chitosan (Kataoka & Ando, 1980b).

The polymerisation of methyl methacrylate and acrylonitrile can be initiated by the copper–chitosan complex and by the copper–glycol chitosan complex in the presence of carbon tetrachloride at neutral pH and at 60°C (Inaki *et al.*, 1978, 1980).

FLOCCULANTS

The well-established use of chitosan as a flocculant for municipal waste waters has been extended to involve the association of chitosan with $AlCl_3$ to aid filtration (Furumori & Okada, 1976) and its association with 3-hydroxy-3,4-dicarboxypentadecanoate to permit electrolysis using a Pt anode and a graphite cathode at 30 V: the suspended matter (1300 ppm) adheres on the anode in a couple of seconds, decreasing the amount in the water to 32 ppm (Inoue, 1977). Kaolin suspensions (2%) have been treated with chitosan (2 ppm) (Furumori & Okada, 1976). Cane sugar molasses have been flocculated with tannic acid and chitosan (Higashikubo & Shinohara, 1977; Ito *et al.*, 1979).

Waste waters containing 2200 ppm starch, at pH 3·6, from rice processing plants were treated with chitosan (5 ppm) and carrageenan (sodium salt (10 ppm)); this resulted in a reduction of the starch content to 35 ppm (Shinoda *et al.*, 1979). Similarly, chitosan has been used to treat aqueous effluent from other food industries including the coagulation of cheese whey solids and suspended matter from both poultry processing and egg breaking plants (Wu *et al.*, 1978).

The potential of chitosan for harvesting algae from stabilisation ponds effluents has been assessed by Rao & Krishnamoorthi (1979) and by Nigam *et al.* (1980): 96·5% of *Scenedesmus acutus* (occurring in water at 1 g litre^{-1}) was flocculated at pH 7·5–8·5 by 0·05 g chitosan litre^{-1}. This method of recovery used 6·3 times less energy than centrifugation and had the additional advantage of avoiding the use of toxic inorganic materials which would prevent the algae being used as an animal feed.

FUNGICIDES

Application to soils

The addition of organic materials to soil to stimulate a flora which is antagonistic to plant pathogens is a promising approach to the biological control of pathogens. Chitin and chitosan possess antimicrobial properties against fungi from specific cell wall composition categories. Chitosan is reported to be more effective than chitin in this respect;

moulds whose cell wall components are cellulose + β-glucan (*Phycomycetes*, *Phytophthora* and *Pythium*), mannan + β-glucan (*Saccharomyces* and *Ascocybe*) and chitin + β-glucan (*Rhizoctonia*, *Datryella*, *Cytospora*, *Cephalosporium*, *Cytosporina*, *Fusarium*, *Verticillium*, *Epidermophyton* and *Basidiomycetes*) do not grow in the presence of chitosan powder (Allan & Hadwiger, 1979). Chitosan applied to *Fusarium*-infected soil stimulates the increase of microorganisms which lyse the fungus (Hatanaka, 1978). Chitin increases the actinomycete population of the rhizosphere of antirrhinum seedlings infected with *Verticillium dahliae*, thus reducing the incidence of antirrhinum wilt (Dutta & Isaac, 1979). The fact that the production of substances inhibitory to the germination of fungal spores by actinomycetes growing on chitin seems almost universal, suggests that antibiotics may be produced in soil containing chitin.

Applications to plants

Chitosan can be directly applied to plants for their protection: a 0·4% chitosan solution sprayed on tomato plants eliminates tobacco mosaic virus infection within 10 days (Ochiai & Kanazawa, 1979). The pea plants protect themselves from *Fusarium solani* f. sp. *pisi*, by responding to the presence of chitosan, a component of the fungal cell wall, and producing phytoalexin, a fungal growth inhibitor. Hadwiger & Beckman (1980) have assayed the production of phytoalexin by applying chitin and chitosan and other carbohydrates to pea pod endocarps and found chitosan to be the most active agent playing a prominent role in the host–parasite interactions.

In fact, the application of *Fusarium solani* f. sp. *phaseoli*, which is non-pathogenic on peas, to pea tissue with or prior to the pathogen *Fusarium solani* f. sp. *pisi*, generates a state of resistance against both fungi. Since the treatment of peas with the non-pathogen protects the plant from the pathogen, chitosan not only mimics the presence of the non-pathogen but also initiates phytoalexin production and inhibits the fungal growth. The action of chitosan on the host which is most beneficial to disease resistance may be one of increasing or maintaining certain enzymes such as phenylalanine ammonia lyase which has the potential to hydrolyse fungal cell walls and to alter the rate of synthesis of proteins as a consequence of the association of chitosan with DNA.

The release of pesticides can be controlled via their incorporation into chitin and chitosan (Wilkins, 1978; Savage *et al.*, 1978).

BIOCHEMISTRY

Increasing interest is being shown in the polysaccharides of fungi; and this has resulted in the use of specific interactions involving chitin and chitosan to identify oligosaccharides.

The oligosaccharide moieties of glycolipids and glycoproteins have gained increased importance with our enlarged understanding of the cell surface receptor mechanism and of cell–cell interactions and recognition. The lectins have been extensively utilised as well as anti-carbohydrate antibodies to describe the concentrations and distributions of the carbohydrate structure on cell surfaces. Antibodies with reactivities to oligosaccharide structures, offer particular advantages for these purposes in that their specificities are usually directed to larger portions of oligosaccharide sequences than the lectins. The anti-oligosaccharide antibodies can be generated by immunisation with oligosaccharides.

The main exocellular polysaccharides of the *Mucorales* (*Absidia cylindrospora*, *Mucor hiemalis* and *Rhizopus nigricans*) have been studied from the immunochemical point of view; they are highly complicated glycans, composed of fucose, mannose, galactose, 2-acetamido-2-deoxy-glucose and 2-acetamido-2-deoxy-galactose. Homogenised emulsions obtained from *Absidia cylindrospora* have been injected into rabbits and antisera have been isolated (Miyazaki *et al.*, 1980).

Similarly, antisera specific for purified cell walls of *Fusarium solani* f. sp. *pisi* and *phaseoli* and for shrimp chitosan were obtained from rabbits and utilised as immunochemical probes to determine the location of fungal components in the pea–*Fusarium* interactions (Hadwiger *et al.*, 1981).

The importance of the ordered conformations of the extra-cellular polysaccharides in determining some immunological properties of bacteria (Muzzarelli, 1977) is shown by studies on the capsular polysaccharides from a serotype of *Escherichia coli* (Moorhouse *et al.*, 1977). The bacterial phage is highly specific for this capsule. Chitin-containing pharmaceuticals have then been prepared for immune

enhancement: chitin (100 g) admixed with starch (400 g) was administered at 1 g day^{-1} to marmots inoculated with HBs antigen (200 μg) and, after 15 days, the antibody value was 38·94 versus 6·89 for the control (Namba *et al.*, 1980).

Antisera with specificities for β(1,4)-N-acetyl-D-glucosamine oligomers have been obtained by immunisation with the oligosaccharides coupled to bovine serum albumin and cytochrome c as carrier proteins. The rabbit antisera reacted with the conjugates. Among those studied the $(GlcNAc)_4$-BSA conjugates competed best in the ^{125}I-$(GlcNAc)_4$-BSA antisera complex formation (Anderas *et al.*, 1979).

Anticholesterolaemics

Many non-metabolisable polysaccharides are effective in reducing the blood cholesterol level in humans and in laboratory animals. It is known that chitosan and its fatty acid complexes inhibit the absorption of cholesterol fatty acids, bile acids and triglycerides from the intestinal tract. For example, trials using rats fed with a cholesterol rich diet for a period of 20 days have shown that both the hepatic and plasmatic cholesterol levels are reduced. The functionality and weight of organs are not affected when chitosan is administered at levels up to 4% of the diet, unlike other materials such as cholestyramine and konjak flour. The effectiveness of chitosan has also been confirmed when compared to alginates, pectates, bentonite and carrageenans (Landes & Bough, 1976; Sugano & Fujikawa, 1978; Kobayashi & Fujikawa, 1979; Kobayashi *et al.*, 1979; Nagyvary *et al.*, 1979; Sugano *et al.*, 1980).

Chitosan and the chitosan–linolenic complex have been incorporated at the level of 1–10% by weight into rye bread, pound cake, salad dressings, soup mixes and pharmaceuticals (Furda, 1980).

Enzyme and food technology

A review article on enzyme immobilisation on chitin and chitosan has recently been published by Muzzarelli (1980a, b, c): most of the enzymes so far immobilised on chitin and chitosan substrates have applications in food technology.

TABLE 2
Enzymes Immobilised on Chitin and Proposed Uses

D-Glucose isomerase: isomerisation of D-glucose to D-fructose
Glucoamylase: conversion of starch to D-glucose
β-D-Galactosidase: hydrolysis of lactose in milk, cheese and whey
β-D-Glucosidase: hydrolysis of cellobiose
D-Glucose oxidase: preparation of D-gluconic acid
AMP deaminase: deamination of AMP to IMP
Urease: conversion of urea to ammonia and carbon dioxide
Papain: removal of haze from beer
Pronase, subtilisin and trypsin: preparation of cosmetics and food proteins
Pepsin: controlled digestion of proteins
Chymotrypsin: plastein synthesis from soya, alfa-alfa and grass
Lysozyme: preparation of pharmaceuticals
Invertase: sugar production
Alkaline phosphatase and acid phosphatase
Escherichia coli cells: synthesis of L-tryptophan from indole and L-serine
Vibrio cholerae: epidemiological studies

A list of these is given in Table 2.

Because of its cationic nature enabling it to react with acidic substances like polyphenols, chitosan was found suitable for the deacidification of fruit, vegetable and coffee extracts. Green and roasted coffee extracts, after electrodialysis and other treatments were treated with chitosan to remove 80% of the free acids without affecting the coffee flavour (Brambilla & Horman, 1980). Similarly, apple juice was deacidifed by contact with chitosan powder for a short time: the product, added to the milk, gave a beverage that did not coagulate; chitosan was regenerated using isopropanol and 1 M KOH (Horman, 1980). Vinegar treated with chitosan did not form a precipitate upon storage (Nakano Vinegar Co., 1980).

Blood anticoagulants

In view of structural similarities between chitosan and heparin, it seems possible that sulphated chitosan could act as a blood anticoagulant: this has been proved experimentally, although the anticoagulant power of these sulphated chitosans is only about 20% of that of heparin. Poly-

electrolytes derived from chitosan and other natural and semi-synthetic polysaccharides have been studied in order to develop compounds useful as blood anticoagulants (Nagasawa, 1976; Kikuchi & Onishi, 1979).

Wound healing acceleration

Chitin and chitosan are better wound healing accelerators than cartilage preparations treated with pepsin. Healing in man is 75% quicker when threads and gauzes treated with powdered chitin derived from lobster or crab are used (Balassa & Prudden, 1978).

Dermatology

Dry shampoos usually contain starch, and this is not completely removed when brushing the hair. A new dry shampoo has been developed containing chitin suspended in alcohol. More recently, a new line of products has been marketed including a lotion and three types of shampoo (Recrin, by Wella) containing 0·5–6·0% of a chitosan salt. These liquid shampoos have the effect of conferring shine and strength to hair due to the interactions between the polysaccharides and the hair proteins (Gleckler & Goebels, 1977; Gross *et al.*, 1977; Konrad *et al.*, 1979; Gross *et al.*, 1980a, b).

SURGERY

To facilitate putting on surgical gloves, talc powder is employed and starch, magnesium oxide or polyglycolic acid have been used as lubricants. In the literature there are many suggestions that such substances cause undesirable reactions if they reach the tissue. In contrast, chitin powder can be absorbed by tissues, is biodegradable, is not toxic nor irritating. Furthermore, it can be easily sterilised by autoclaving or by treatment with ethylene oxide vapour since it does not clot or hydrolyse during such treatments. Chitin, because it can be degraded by lysozyme, can with impunity contact skin and wounds, if anything accelerating healing. Furthermore, its moisture absorbing capability ensures the best tactile conditions. Similarly, 6-O-carboxymethyl chitin, 6-O-(2′-

hydroxyethyl)chitin and 6-O-ethyl chitin are useful in these applications (Casey, 1977, 1978). Suture threads made out of chitin, have great mechanical strength, can be stored for long periods, can be sterilised in the traditional way, can be dyed and can incorporate medicaments; above all, they have the characteristic of being degraded by tissues after a sufficiently long period.

The absorption *in vivo* is caused by lysozyme which is present in all biological fluids: chitin susceptibility to lysozyme can be altered by chemical modification of the chitin. Fibres of chitin and of modified chitin can be stored in normal environmental conditions and do not require to be protected from moisture though their flexibility increases when they are wetted as does catgut. It is possible to use gauzes, sponges and bandages woven with chitin threads and also to make stiffer objects such as haemostatic tweezers (Capozza, 1976a, b; Austin, 1979).

N-ACETYL GLUCOSAMINE AND GLUCOSAMINE IN MEDICINE

Glucosamine salts which are easily obtained from chitin have several medical applications. The use of glucosamine hydrochloride in gastroenterology has been known for a few years as an alternative to the corticosteroid treatment of enteritis and ulcerative colitis (Prudden, 1976) and the antitumour activity of the acetylated hexosamines has been demonstrated recently. Fluorinated and acetylated glucosamines have been tested to determine their effects on glucosamine and leucine incorporation into glycoconjugates: the most cytotoxic agent was found to be 2-acetamido-2-deoxy-1,3,4,6-tetra-O-acetyl-β-D-glucopyranose (Bernacki *et al.*, 1977). D-Glucosamine itself is toxic to certain malignant cell lines and tumours *in vivo* at concentrations that have little effect upon normal host tissues (Friedman & Skehan, 1980) and therefore has uses in cancer chemotherapy. D-Glucosamine is an inhibitor of the glycosylation of the viral envelope and decreases the rate at which tumours are induced in animals by Rous sarcoma virus and is active against the human influenza virus (Floc'h & Werner, 1976; Friedman, 1979). Research on rheumatology has also shown that osteoarthritis and similar diseases can be efficiently treated with the aid of glucosamine salts (Dettmer, 1979; Drovanti *et al.*, 1980; Vajaradul,

1981). The salt of glucosamine and c-AMP can be used for the treatment of heart hypertrophy and muscular metabolism alterations (Reiner & Rossi, 1975).

CONCLUSIONS

Substantial progress has been made during the period 1977–80, following the publication of the book by Muzzarelli (1977). Much of this has been along the lines indicated at the First International Conference on Chitin and Chitosan (Muzzarelli & Pariser, 1978).

Chitosan has been shown to be a reactive polysaccharide (Muzzarelli, 1978a) and many chitosan derivatives have been produced and characterised. The carboxymethyl derivatives (including the 'old' O-carboxymethyl chitosan and the novel N-carboxymethyl chitosan (Muzzarelli *et al.*, 1982e) whose potential was stressed by Muzzarelli (1977, page 122), have attracted much attention because of their unique properties (Koshugi, 1980a, b, c, 1981a, b; Mrachkovskaya *et al.*, 1981; Muzzarelli, 1981a).

The variety of chitosan derivatives obtained from aldehydes and acyl chlorides (crosslinked chitosan copolymers (Berkovich *et al.*, 1980), fully acylated chitosans (Fujii *et al.*, 1980) and porous acylated chitosans (Koshugi, 1981c)) are examples of the diverse range of products that can be obtained from just one approach. An aldehyde (salicylaldehyde) derivative of chitosan (other than glutaraldehyde) was first described by Plisko *et al.* (1972); and the importance of the aldimines was emphasised by Muzzarelli (1977, page 132, and 1978b) and confirmed by recent work by Hirano *et al.* (1979), Hall & Yalpani (1980), Moore & Roberts (1981) and Muzzarelli *et al.* (1982b, c).

It is now evident that chitin can be dissolved in a choice of solvents, spun into fibres or reacted in homogeneous phase, whilst not long ago it was considered as an 'intractable polymer'.

Allan *et al.* (1978a) realised that it would be possible to obtain chitin not only from crustaceans (shells from canning plants) but also from fungi (mycelia from industrial fermentations); indeed, there has been a considerable amount of research on chitin from this source.

Earlier ideas have been extended; for instance, the use of lithium salts originally proposed by Von Veimarn (1927) has been applied to chitin (Rutherford & Austin, 1978a); the potential of alkylglycosides of

glucosamine demonstrated by Gyorgy *et al.* (1954) has been confirmed by Austin *et al.* (1981), Austin & Reed (1979), Austin & Reed (1981), Dunn & Farr (1979). The importance of chitosan in the study of fungicides, anticipated by Lingappa & Lockwood (1962) has been confirmed in recent studies by Dutta & Isaac (1979) and Hadwiger *et al.* (1981) while the potential of alkali chitin first discussed by Thor & Anderson (1940) and noted by Muzzarelli (1978b) has now been utilised by the Mitsubishi Rayon Co. (1980a, b, c).

Enzyme immobilisation is a rapidly growing field particularly with respect to applications in the food industry. Despite fears about the use of chitin as a food additive it has now been demonstrated that the ingestion of reasonable quantities of chitin/chitosan is not only safe but may be beneficial. An edible food-wrapping film made of starch and chitosan has been proposed by the Kuraray Co. (1980). It has been shown that the administration of chitin to the human body in various ways (medicaments, shampoos, healing bandages, food additives and many others) can be of benefit. Thus, we may foresee more research projects being carried out in the near future, leading to the inclusion of chitin in more cosmetics, pharmaceuticals and surgical aids, besides clothes (Babu *et al.*, 1975).

The adsorption capacities of chitin/chitosan give rise to applications in various fields: removal of toxic gases (Sanyo Kokusaku Pulp Co., 1980), activation of carbon (Dainichiseika Color & Chemical Mfg. Co., 1980b) removal of dyes (Shinagawa *et al.*, 1980) and removal of petroleum (Safronova, 1980). Chitosan is also being used in more elegant and refined studies, dealing, for instance, with molecular recognition, immunochemistry, glycoprotein chemistry.

The oligosaccharides of chitin, well known for their uses in the elucidation of the lysozyme structure, and the glucosamine salts are gaining widespread applicability in medicine for a number of purposes.

The fundamental aspects of the research on chitin are beyond the scope of this review; it should be said in any case that basic research is progressing at the same pace as applied research and therefore, proposals for further applications are expected to stem from the novel information now being gained on biosynthesis, crystallinity, association with other polysaccharides, etc.

Chitin is no longer an intractable substance nor is chitosan a laboratory curiosity: today, these carbohydrate polymers are important renewable resources.

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