Proc., Symp. on Advances and Priorities in Fisheries Technology, SOFT(I) and CIFT Cochin, 11-13 Feb: 210 (1998)

Emerging Trends in the Application of Chitin and Chitosan

P.T. MATHEW, K.G. RAMACHANDRAN NAIR and P. MADHAVAN
Central Institute of Fisheries Technology, Cochin 682 029

Chitin and derivatives have applications in clarification and purification, chromatography, paper, textiles, food and nutrition, photography, medicine, pharmaceuticals, cosmetics, agriculture, enzyme immobilisation, fibres, films, membranes etc. Chitosan is a biodegradable cationic polyelectrolyte which can be used as haemostatic agent, wound dressing, membrane for dialysis, anticholesterolemic dietary supplement and in controlled delivery of drugs. It is a lipid binding food additive. Oral administration of chitosan leads to partial hydrolysis and generation of D glucosamine. A review of the emerging trends in the applications is made in this paper.

Key words: Chitin, chitosan, applications

Chitin, polymer of N-acetyl glucosamine, is present in the exoskeleton of shrimp, krill, crab, squilla and insects, squid pen, fungi etc. The major commercial source of chitin is the crustacean waste, mainly of shrimp and crab. Shrimp waste contains about 5% chitin and crab waste about 3%. Chitin is isolated from crustacean shell by deproteinisation using dilute alkali or proteoloytic enzymes followed by demineralisation using dilute mineral acids. Chitin is deacetylated using concentrated alkali to produce chitosan. Properties of chitin and chitosan vary considerably depending on the raw materials and the process employed (Madhavan and Nair, 1974; Madhavan, *et. al.*, 1986).

Properties

Most commercial polysaccharides are neutral or acidic. Though chitin is neutral, chitosan is basic. Muzzarelli (1977) reviewed in detail the occurrence, structure, properties and applications of chitin and chitosan. Their unique properties include polyelectrolyte behaviour, polyoxysalt formation, film formation and chelation of metal ions. Chitin is highly hydrophobic and insoluble in water and most organic solvents. It is soluble in hexafluro isopropanol, hexafluro acetone and chloroalcohols in conjunction with aqueous solutions of mineral acids. Chitin on hydrolysis with concentrated acids yields relatively pure amino sugar, D glucosamine (Radhakrishnan and Prabhu, 1971). Rutherford and Austin (1978) determined the intrinsic viscosity of crab chitin and shrimp chitin and computed the molecular weight as 0.4×10^6 to 1.8×10^6. Molecular weight of shrimp chitin has been reported as 1.5×10^5 (Anon, 1991).

Applications of Chitin and Chitosan

Chitin, chitosan and their derivatives find extensive applications in various fields (Madhavan, 1992). These include clarification and purification, chromatography, paper and textiles, photography, food and nutrition, agriculture, pharmaceutical and medical, cosmetics, biodegradable membranes and biotechnology.

Clarification and purification

Chitosan in solution is a good coagulant. It can wrap solid particles in liquids, bring them together and agglomerate. This property is applied to remove suspended solids in waste water from food processing plants and in clarification of beverages. The food related applications are drinking water, clarification of fruit juices and recovery of microalgae (Bough and Landes, 1978). Chitosan is a better clarifying agent than potash alum for contaminated water (Prabhu *et. al.,* 1976). The non-food related applications are in treatment of sewage effluents, sand and gravel wash, metal finishing, electroplating wastes, and radioactive wastes. It can adsorb transition metals. Heavy metal ions in water could be selectively concentrated by ultra filtration associated with complex formation by means of chitosan (Taha *et. al.,* 1996). Martel *et. al.* (1996) transformed a chitosan based non-woven fabric filter into a strong cation exchanger by grafting a mono or di-sulphonic aromatic group for sorption of lead and chromium.

Chromatography

Free amino and hydroxyl groups in chitosan make it a good chromatographic support. Chitosan could be used in thin layer chromatography for separation of nucleic acids (Nagasawa *et. al.,* 1971). Novel composite materials like chitosan-$CuCl_2$ chelate membranes were prepared and a method was developed for qualitative detection of carbonate ions by FT-IR spectra using this membrane (Hirano *et. al.,* 1996).

Paper and textiles

Chitosan improves the bursting strength, puncture resistance, water proofness, tensile strength and water vapour transmission rate of Kraft paper (Gopal *et. al.,* 1981). Kobayashi *et. al.,* (1982) applied chitosan acetate to improve the surface strength of 'hosho paper'. Chitin and chitosan can be used for production of fibres and films. Wei and Hudson (1994) prepared a chitosan-sodium dodecyl sulphate fibre composed of hydrophobic microdomains, which can solubilise water insoluble dyes. This chitosan-SDS three dimentional net work may be of use in controlled release applications.

Because of its chelating ability, adhesive property and ionic bond forming characteristics, chitosan finds application in textiles. Fabrics sized with chitosan have good stiffness, improved dye uptake, added lustre and improved laundering resistance. Sizing with chitosan is permanent and the coating formed is insoluble in water.

Photography

Due to its resistance to optical characteristics, film forming ability and reactions with silver complexes, chitosan finds applications in photography. Silver complexes are not appreciably retained by chitosan and, therefore, can easily penetrate from one layer to another by diffusion transfer.

Food and nutrition

The presence of chitin in marine invertebrates, insects, fungi, yeast and cell walls of certain plants and of chitosan in various fungi indicates that chitin and chitosan are already part of our food supply. Chitosan is nontoxic to mice when fed at 18g or less free chitosan/kg body weight/day (Arai *et. al.*, 1968). Toxicity studies showed that LD50 value of chitosan is greater than 16g/kg body weight and it is as safe as salt and sugar (Asano *et. al.*, 1978). Chitin is used as an ingredient in domestic animal feeds and develops no abnormal symptoms in animals fed on chitin containing feeds (Hirano *et. al.*, 1990). It is a good growth promoter in broiler chicks, pigs and rabbits (Nair *et. al.*, 1987, 1993). Chitosan is a thickening and stabilising agent in foods and enhances the storage life of meat (Geonghea, 1994). Emulsifying properties of chitin, its potential as a non-absorbable carrier for food dyes and as a protein coagulant aid to recover protein from food processing waste were reported by Knorr (1984).

Chitosan is hypolipidemic and hypocholesterolemic (Mathew *et. al.*, 1995). Serum cholesterol, triglycerides and free fatty acids decreased in domestic animals when fed on high fat diet containing 1.2-1.4 g chitin/kg of body weight (Hirano *et. al.*, 1990). Chitosan is capable of binding fatty acids by forming corresponding complex salts not hydrolysed by hydrochloric acid in the stomach. These salts bind additional lipids, cholesterol and other sterols and escape through excreta (Furda, 1983). Glucosamine is ineffective whereas chitosan is effective in the hypocholesterolemic action showing that certain degree of polymerisation is required for lowering cholesterol level (Sugano, *et. al.*, 1992). Maezaki *et. al.* (1993) found that the serum total cholesterol level significantly decreased in adult men while the serum HDLO cholesterol significantly increased and resulted in a significant decrease in the atherogenic index.

Oral administration of chitosan leads to partial hydrolysis and generation of the monomer, glucosamine. Current studies indicate that there is reasonable, though incomplete, evidence that digestive enzymes, mainly pancreatic lipase, are able to hydrolyse chitosan. Generation of glucosamine from chitosan by human digestive enzymes and its role in various enzymatic activities like inhibition of glucokinase are subjects which need further research (Muzzarelli, 1996).

Agriculture

Chitin and derivatives control nematodes during germination and culture of seeds, enhance protection against pathogenic organisms in plants and suppress them in soil, induce chitinase activity, proteinase inhibitor synthesis and antiviral activity, and encapsulate fertilisers for controlled release of herbicides (Hirano *et. al.*, 1989). Chitinous materials are efficient to control parasitic nematodes in ornamental plants, cucumber and tomato (Brown *et. al.*, 1982). When rice callus was treated with water soluble N-acyl (C_2-C_5) derivatives of carboxymethyl chitosan as elicitors stimulated phenylalanine ammonia-lyase activity two-fold, chitinase activity 3.5 fold and lignification 1.7 fold (Notsu *et. al.*, 1994). Coating with chitosan delayed ripening process in tomato, bell pepper and strawberry (Arul and El Ghaouth, 1996), mango and banana (Anon, 1988) and maintained the quality attributes.

Medical and pharmaceutical

Chitin and derivatives can be employed as bacteriostatic agent (Malette et. al., 1983); anticholesterolemic agent (Mathew *et. al.*, 1995), drug delivery vehicle (Sujatha, 1990), spermicide (Smith, 1984), enzyme immobiliser (Mathew, 1989; Synowiecki *et. al.*, 1994), membrane for dialysis (Claramma *et. al.*, 1988), haemostatic agent, and in contact lens, surgical glove powder, hydrogel ointment base, antisore compositions, wound dressing (Allan *et. al.*, 1984; Radhakrishnan *et. al.*, 1991), treatment of stomatitis prothetica mycotica (Knapczyk *et. al.*, 1994) and leg ulcers (Protas-Drozd and Gwiezdzinski, 1994). Acetylated glucosamine has been found effective in treatment of tumour cells. Osteoarthritis can be effectively treated with glucosamine salt (Dettmer, 1979). Chitin fibre has all the characteristics of an ideal surgical suture such as biodegradability, resistance to alkali and digestive system, softness, knot reliability, wound healing acceleration and tissue adaptation. These make it useful in digestive tract and urological surgery (Nakajima *et. al.*, 1984). Another use of chitin is in making temporary artificial ligaments for the knee joint and materials for less rigid fixation (Maida *et. al.*, 1984). Applications of chitosan in dentistry also have been reported (Sapelli *et. al.*, 1989; Praveen, 1990; Mani and Cheru, 1991). Chitosan ascorbate in powder form could be applied as haemostatic and protective dressing in oral cavity during dentistry and prosthetic surgery (Kochanska *et. al.*, 1994).

Cosmetics

Chitosan coated liposome retains water at a higher rate than naked liposomes (Onsoyen, 1992). The efficacy of Hydagen CMF, a 1% solution of special grade chitosan in 0.4% glycolic acid was evaluated in different formulae in both skin and hair care applications. It improved skin sensation and reduced irritation potential. Hydagen CMF film is homogenous and highly flexible and shows no cracks or scaling in the dry state (Wachter and Steinberg, 1996).

Enzyme immobilization

An important application of chitin is in immobilization of enzymes (Muzzarelli, 1977). Mathew (1989) used chitosan in immobilizing glucose 6-phosphate dehydrogenase from fish. The most common method of fixing enzyme to chitosan is by cross-linking with dialdehydes like glyoxal and glutaraldehyde. Chandy and Sharma (1992) reported poly lysine-immobilized chitosan beads as adsorbant for bilirubin. Chitin activated by 5 M potassium hydroxide solution and treated with 4% glutaraldehyde is a suitable support for immobilization of chymosin and seal gastric protease (Synowiecki *et. al.*, 1994).

Fibres, films and membranes

Chitosan films are clear, tough, flexible and good oxygen barriers. Chitosan based coatings can protect foods from fungal decay. However, these are not yet approved as food ingredients in the USA (Krochta and Johnston, 1997). Edible and biodegradable polymer films offer alternative environment friendly packaging.

Production of high strength films and fibres of chitin/chitosan has been made possible with the development of non-degrading solvent system. For films chitosan dissolved in weak organic acids, about 2% acetic or formic, is cast onto a smooth surface. The anion from the film is removed by heating at 90°C or by treating the dried film with alkali. Microcrystalline chitin/chitosan (Austin *et. al.,* 1981) is used for direct film forming. Chitin fibre has been prepared from chitin viscose (Noguchi *et. al.,* 1978).

Membrane of chitin/chitosan is produced by casting their solutions either alone or with a suitable polymer and other reagents to give desired properties (Blair *et. al.,* 1987). A chitosan collagen bipolymer matrix was used for studying the cell growth rate by Izume *et. al.* (1989). Albumin blended chitosan membrane can be used in haemodialysis, artificial skin and drug targeting (Chandy and Sharma, 1987). Blair *et. al.* (1987) studied tensile strength, elongation and hydrophilic properties of chitosan and polyvinyl alcohol blended membranes made from crab shell. A classical polyelectrolyte complex is formed between the anionic sites of collagen and cationic sites of chitosan when chitosan interacts with collagen (Taravel and Domard, 1994).

Conclusion

Though chitin is one of the most abundant natural polymers having wide and varied potential applications in several fields, no serious attempts were made in exploring these possibilities until the late seventies. One of the best known applications is in clarification and purification. Increased knowledge on the properties of chitin led to the development of its various derivatives having many high value applications, some of which are in areas like medicine, agriculture and cosmetics.

References

Allan, G.G., Altman, L.C., Bensinger, R.E., Ghosh, D.K., Hirabayashi, Y., Neogi, A.N. & Neogi, S. (1984) in *Chitin, Chitosan and Related Enzyme* (Zikakis, J.P., Ed.) p. 119, Academic Press, Inc., Orlando

Anon (1988) *Annual Report* 1987-88, p. 29, Central Institute of Fisheries Technology, Cochin

Anon (1991) *Annual Report* 1990-91, p. 11, Central Institute of Fisheries Technology, Cochin

Arai, K., Kinumari, T. & Frigota, T. (1968) *Bull. Tokai Reg. Fish. Lab.,* **56**, 889

Arul, J. & El Ghaouth, A. (1996) in *Advances in Chitin Science,* Vol. I (Domard, A. Jeuniaux, C., Muzzarelli, R. & Roberts, G., Eds) p. 372, Jacques ANDRE, France

Asano, T., Hawakawa, N. & Suzuki, T. (1978) in *Proc. First Int. Conf. Chitin/Chitosan,* (Muzzaralli, R.A.A. & Pariser, E.R., Eds) p. 231, MIT, Cambridge

Austin, P.R., Brine, C.J., Castle, J.E. & Zikakis, J.P. (1981) *Science,* **212**, 749

Blair, H.S., Guttric. J., Law, T. & Turkington, P. (1987) *J. Appl. Poly. Sci.,* **33**, 641

Bough, W.A. & Landes, D.R. (1978) in *Proc. First Int. Conf. Chitin/Chitosan,* (Muzzarelli, R.A.A. & Pariser, E.R., Eds) p. 218 MIT., Cambridge

Brown, L.R., Brown-Skrobot, S., Teichert, C., Blasingame, D.G. & Ladner, C.M. (1982) in *Chitin and Chitosan,* (Hirano, S. & Tokura, S., Eds) p. 227, The Japanese Society of Chitin and Chitosan, Tottori University, Japan

666.max

Chandy, T. & Sharma, C.P. (1987) *Polym. Mat. Sci. Engg.*, **57**, 299

Chandy, T. & Sharma, C.P. (1992) *Artificial Organs*, **16**(6), 568

Claramma, C.V., Chandy, T., Sharma, C.P. (1988) in *Proc. The Third World Biomaterial Congress*, p. 477, Kyoto, Japan,

Dettmer, N. (1979) *Z. Rheumatol.*, **38**, 163

Furda, I. (1983) in *Unconventional Sources of Dietary Fibre* (Furda, I., Ed.) American Chemical Society, Washington, DC

Geonghea, W. (1994) *Chemical Abstracts, Abs.* No. 75820, **120**

Gopal, T.K.S., Nair, K.G.R., Thankappan, T.K. & Govindan, T.K. (1991) *Perfect. Pac.* **21** (8&9), 58

Hirano, S., Hayashi, M., Nishida, T. & Yamamoto, T. (1989) in *Chitin and Chitosan*, (Skjak-Braek, G., Anthonsen, T. & Standford, P., Eds) p. 743, *Elsevier Applied Science, London*

Hirano, S., Itakura, C., Seino, H., Akiyama, Y., Nonaka, I., Kanbara, N. & Kawakami, T. (1990) *J. Agric. Food Chem.*, **38**, 1214

Hirano, S., Yamamoto, K., Yamada, M., Inui, H. & Ji, M. (1996) in *Advances in Chitin Science*, Vol. 1 (Domard, A. Jeuniaux, C., Muzzarelli, R.A.A. & Roberts, G., Eds) p. 137, Jacques ANDRE, France

Izume, M., Kimura, T.T. & Miata, T. (1989) in *Chitin and Chitosan*, (Skjak-Braek, G., Anthonsen, T. & Standford, P., Eds) p. 653, *Elsevier Applied Science, London*

Knapczyk, J., Majewski, S., Pawlik, B. & Wisniewska, G. (1994) in *Chitin World*, (Karnicki, Z.S., Wojtasz-Pajak, A., Brzeski, M.M. & Bykowski, P.J., Eds) p. 504, Wirtschaftsverlag, NW, Poland

Knorr, D. (1984) *Food Technology*, **38**, 85

Kobayashi, Y., Nishiyama, M., Matsuo, R., Tokura, S. & Nishio, N. (1982) in *Chitin and Chitosan*, (Hirano, S. & Tokura, S., Eds) p. 239 The Japanese Society of Chitin and Chitosan, Tottori University, Japan

Kochanska, B., Witek, E. & Sramkiewicz, J. (1994) in *Chitin World*, (Karnicki, Z.S., Wojtasz-Pajak, A., Brzeski, M.M. & Bykowski, P.J., Eds) p. 520, Wirtschaftsverlag, NW, Poland

Krochta, J.M. & Johnston, C.M. (1997) *Food Technol.*, **51**(2), 60

Madhavan, P. & Nair, K.G.R. (1974) *Fish. Technol.* **11**, 50

Madhavan, P. (1992) *Chitin, Chitosan and Their Novel Applications*, Popular Science Lecture Series, Central Institute of Fisheries Technology, Cochin

Madhavan, P., Nair, K.G. R., Thankappan, T.K., Prabhu, P.V. & Gopakumar, K. (1986) *Production of Chitin and Chitosan*, Project Report, Central Institute of Fisheries Technology, Cochin

Maezaki, Y. Tsujio, K. & Nakagawa, Y. (1993) *Biosci. Biotech. Biochem.*, **57**, 1439

Maida, M., Inoue, Y., Iwase, H. & Kifune, K. (1984) in *Chitin, Chitosan and Related Enzymes* (Zikakis, J.P. Ed.) p. 411, Academic Press, Orlando

Malette, W.G., Quingley, H.J., Gaines, R.D., Johnson, N.D. & Rainer, W.G. (1983) *Ann. Th. Suzg*, **36**, 55

Mani, K. & Cheru, R.T. (1991) M. D. S. Dissertation, University of Kerala, Trivandrum

Martel, B., Weltrowski, M., Morcellet, M. & Scheubel, G. (1996) in *Advances in Chitin Science*, Vol. 1 (Domard, A., Jeuniaux, C., Muzzarelli, R.A.A. & Roberts, G., Eds) p. 291, Jacques ANDRE, France

Mathew, P.T. (1989) *Purification and Characterisation of Glucose 6-Phosphate Dehydrogenase in Fish*, Ph.D. Thesis, Cochin University of Science and Technology, Cochin

Mathew, P.T., Nair, K.G.R. & Madhavan, P. (1995) in *Proc. Working Party on Fish Technology and Marketing,* FAO Fisheries Report, No. 514, p. 265, Food and Agriculture Organisation of the United Nations, Rome

Muzzarelli, R.A.A. (1977) *Chitin,* Pergamon Press, Oxford

Muzzarelli, R.A.A. (1996) in *Advances in Chitin Science,* Vol. I (Domard, A., Jeuniaux, C., Muzzarelli, R.A.A. & Roberts, G., Eds) p. 448, Jacques ANDRE, France

Nagasawa, K., Watnabe, H. & Orgamo, A. (1970) *J. Chromatogr.,* **47**, 408

Nagasawa, K., Watnabe, H. & Orgamo, A. (1971) *J. Chromatogr.,* **56**, 378

Nair, K.G.R., Mathew, P.T., Madhavan, P. & Prabhu, P.V. (1993) *Fish. Technol.,* **30**, 24

Nair, K.G.R., Mathew, P.T., Madhavan, P. & Prabhu, P.V. (1987) *Indian J. Poult. Sci.* **22** (1), 40

Nakajima, M., Atsumi, K. & Kifune, K. (1984) in *Chitin, Chitosan and Related Enzymes* (Zikakis, J.P., Ed.) p. 407, Academic press, Orlando

Noguchi, J., Tokura, S. & Nishi, N. (1978) in *Proc. First Int. Conf. Chitin/Chitosan* (Muzzarelli, R.A.A. & Pariser, E.R., Eds) p. 315, MIT., Cambridge

Notsu, S., Saito, N., Kosaki, H., Inui, H., & Hirano, S. (1994) in *Chitin World* (Karnicki, Z.S., Wojtasz-Pajak, A., Brzeski, M.M. & Bykowski, P.J., Eds) p. 229, Wirtschaftsverlag, NW, Poland

Onsoyen, E. (1992) in *Advances in Chitin and Chitosan* (Brine, C.J., Sandford, P.A. & Zikakis, J.P., Eds) p. 479, Elsevier Applied Science, London

Prabhu, P.V., Radhakrishnan, A.G. & Iyer, T.S.G. (1976) *Fish. Technol.,* **13**, 69

Praveen, B. (1990) M.D.S. Dissertation, M.G.R. Medical University, Chennai

Protas-Drozd, F. & Gwiezdzinski, Z. (1994) in *Chitin World* (Karnicki, Z.S., Wojtasz-Pajak, A., Brzeski, M.M. & Bykowski, P.J., Eds) p. 590, Wirtschaftsverlag NW, Poland

Radhakrishnan, A.G. & Prabhu, P.V. (1971) *Res. Ind.,* **16**, 265

Radhakrishnan, A.G., Vijayan, V.V., Sambasivan, M.S., Jamaluddin, M. & Rao, B.S. (1991) *Biomedicine,* **2**, 3

Rutherford, F.A. & Austin, P.R. (1978) in *Proce. First Int. Conf. Chitin/Chitosan* (Muzzarelli, R.A.A. & Pariser, E.R., Eds) p. 182, MIT, Cambridge

Sapelli, P.L., Baldassarre, V., Muzzarelli, R.A.A. & Emanuelli, M. (1989) in *Chitin in Nature and Technology,* (Muzzarelli, R.A.A., Jeuniaux, C. & Gooday, G.W., Eds) p. 507 Plenum Press, New York

Smith, R.L. (1984) US Patent, 4474769

Sugano, M., Yoshida, K. Hashimoto, M. Enomoto, K. & Hirano, S. (1992) in *Advances in Chitin and Chitosan* (Brine, C.J., Sandford, P.A. & Zikakis, J.P., Eds) p. 472, Elsevier Applied Science, London

Sujatha, N. (1990) M. Pharm Thesis, University of Kerala, Trivandrum

Synowiecki, J., Han, X.Q. & Shahidi, F. (1994) in *Chitin World,* (Karnicki, Z.S., Wojtasz-Pajak, A., Brzeski, M.M. & Bykowski, P.J., Eds) p. 555, Wirtschaftsverlag, NW, Poland

Taha, S., Bouvet, P., Corre, G. & Dorange, G. (1996) in *Advances in Chitin and Chitosan,* Vol. (1) (Domard, A., Jeuniaux, C., Muzzarelli, R.A.A. & Roberts, G., Eds) p. 389, Jacques ANDRE Publisher, France

Taravel, M.N. & Domard, A. (1994) in *Chitin World* (Karnicki, Z.S., Brzeski, M.M., Bykowski, J & Wojtasz-Pajak, A., Eds) p. 462, Wirtschaftsverlag NW, Poland

Wachter, R & Steinberg, E. (1996) *Advances in Chitin Science,* (Domard, A., Jeuniaux, C., Muzzarelli, R.A.A. & Roberts, G., Eds) Vol. I, p. 381 Jacques ANDRE Publisher, France

Wei, Y.C. & Hudson, S.M. (1994) in *Chitin World* (Karnicki, Z.S., Brzeski, M.M., Bykowski, J. & Wojtasz-Pajak, A., Eds), p. 190, Wirtschaftsverlag NW, Poland

666.max