MEETING REPORT

Coming of age: Chitin chitosan research*

More than 200 years ago, Henri Braconnot, a French chemist, described chitin in mushrooms. It took about a hundred years for Albert Hofmann in Switzerland to decipher the structure of chitin from the snail, *Helix pomatia.* Chitin, a long chain polymer of *N*-acetylglucosamine, is found in the exoskeletons of insects and crustaceans such as crabs, lobsters, shrimps, in the mouth parts of most molluscs, cell walls in fungi, scales of fish and on certain amphibians and nematodes. As a polysaccharide, it is second only to cellulose, in terms of abundance.

In recent decades, humans who, by the way, do not produce this polysaccharide in their body, woke up to the uses of chitin and its deacetylated version, chitosan, in agriculture, in environmental technologies, in industry, in medicine...

Given the huge coastal belts of India where marine fisheries bring out tonnes of chitin from the sea – which becomes a nuisance if not attended to – scientists and researchers interested in the subject came together and formed the Indian Chitin and Chitosan Society, eight years ago. The 7th meeting of the Society was held at the CSIR-National Chemical Laboratory, Pune from 11 to 13 October 2018.

Rajendra Prasad, Amity University, Haryana, the chief guest, initiated the discussion starting with the problem of fungal infections. Fungal infections are more difficult to treat than bacterial infections and fungi seem to develop drug resistance as fast as, perhaps even faster, than bacteria. Chitin is an obvious target for designing anti-fungals. Rajendra Prasad gave a brief description of his work on targeting the molecules of the fungal cell wall, especially chitin.

Later in the meeting, T. Sathyanarayana, Delhi University, presented the possibility of sourcing thermostable chitinases from thermophilic moulds to overcome the problem of the stability of the enzyme that breaks down chitin. He put forth a case study of the mould, *Myceliophthora thermophilia*, where his lab had explored the enzyme in detail, including cloning the gene to enable mass production. They found that it is effective against fungi, insects and nematodes.

In the first session, Mukund Deshpande, the convener of the meeting at the NCL Pune, gave a brief account of enzymes that break down chitin. He pointed out that the breakdown of chitin is an important physiological function in fungi, insects and plants and there are specialized enzymes that they produce for growth, development and as defence as well as for attack. He gave a brief account of the use of chitinases and chitosanases in the laboratory, in industries, in health and in agriculture. Using examples, he made a distinction between the low cost, high volume required for agriculture and the high cost, low volume for medical and laboratory applications in the production of these enzymes and pointed out some trajectories for further research.

Chitosan became the focus of discussion in the meeting. It was pointed out that chitosan is a generic term that includes polymers with a varied number of subunits with a range of deacetylation. The need to evolve more specific terminologies to describe the molecules used in the experiments was echoed by many in the meeting.

Chitosan in agriculture

J. M. Rajwade, Agharkar Research Institute, Pune reported how their lab established the usefulness of the foliar application of zinc-complexed chitosan nanoparticles on wheat to improve micronutrient availability from the grains produced. The NH_2 group of chitosan can be used to attach zinc and the stomata of the leaf takes up the chitosan-zinc nanoparticles. Foliar spray at the flowering stage of wheat can reduce zinc deficiency in our populations.

S. G. Dalvi, Vasantdada Sugar Institute, Pune pointed out the high cost and time for enzymatic degradation of polysaccharides and suggested irradiation of chitosan to improve its bioactivity. Testing the concept for seed priming and foliar spray in sugarcane, he showed that the method is superior in antiviral, antifungal activities as well as for nutrient uptake and growth of the plant. Besides improving sugarcane yield, it improves the performance of intercrops such as onion and peanuts. He also stressed the importance of chitosan for the bioremediation of sugar industry effluent.

K. V. Harish Prashanth, CSIR-CFTRI, Mysuru, pointing out the increase in the market for chitosan and the phenomenal increase in research on the polymer, warned researchers about potential pitfalls. Lack of reliable statistics, collaborative research efforts between academia and industry and market research related to the ultimate customers, as well as lack of funding, pose a threat to the growth of the chitosan market. Innovative research is needed to tap new opportunities. He cited the example of using chitosan mixed with rayon to produce textiles for weak and sensitive skin.

The annual production of chitin is around 100 billion tonnes. That does not mean that we have to use it all up in agriculture. Subha Narayan Das, Indira Gandhi National Tribal University, pointed out how the amount of chitosan used for plant protection has decreased from 40 kilograms to just 4 grams per hectare. It might even decrease further. The degree of polymerization and acetylation as well as the pattern of acetylation influence the bioactivity of chitosan.

J. Madhuprakash, University of Hyderabad, said that chitooligosaccharides for various purposes can be made by chemical and enzymatic methods. Chemical means are quite often accompanied by pollution. The choice of enzymes as ecologically safer alternatives is very large. For example, *Serratia proteamaculans*, an endophytic bacteria, produces four different types of chitinases on chitin-rich media. One of them can be used for transglycalation – transfer of subunits from one polymer to another. Through a series of mutations and selections, they developed a process for the large-scale production of chitooligosaccharides with specific acetylation sites, and higher activity.

*A report on the 7th Indian Chitin and Chitosan Society Meeting, held at CSIR-NCL, Pune from 11 to 13 October 2018.

Chitosan in health and medicine

Vandana Ghormade, Agharkar Research Institute, Pune enumerated the properties of chitosan that make it a polymer of choice in many medical applications. Chitosan is mucoadhesive, has high permeability; it is biocompatible, biodegradable, non-antigenic, non-toxic... Polymeric chitosan nanoparticles are stable, entrap drugs efficiently and are released in a slow, sustained manner. The nanoparticles have large surface area for easy attachment and fast mass transfer and can thus be used to deliver not only drugs but also plasmids, genes and small oligonucleotides, and proteins. They are easy to synthesise with various options: ionic gelation (for insulin, BSA, cyclosporine A, rifampicin), coacervation (for DNA), precipitation emulsion (for gadopentetic acid), coalescence reverse micellar (fordoxorubicin)... The cadmium–tellurium quantum dots embedded in chitosan nanoparticles make them almost non-toxic and they are used in bioimaging. However, size heterogeneity and dispersity of polymeric nanoparticles affect their overall charge, loading efficiency and controlled drug release, she pointed out. The microfluidic method offers controlled movement of reactants and their reaction in precise reduced volumes to yield monodispersed nanoparticles. Drug loading was high and release was slow and sustained in the case of chip synthesized nanoparticles. Coating of magnetic nanoparticles for improved dispersibility and drug loading has been used in breast cancer to combine pharmacological and thermal treatments. However, only chitosan wound dressings have FDA approval. The source, the characteristics and purity of chitosan are factors that need focused attention from researchers.

N. Selvamurugan, S.R.M. Institute of Science and Technology, talked about experiments in bone tissue regeneration. His team designed different types of scaffolds that incorporate chitosan nanoparticles. They loaded the chitosan nanoparticles with microRNAs, known to be involved in bone tissue differentiation, and with different phytochemicals that have osteogenic properties and demonstrated the value of using such approaches to regenerate tissues.

J. Venkatesan, Yenepoya Research Center, Mangaluru used chitosan-single walled CNT composites and checked chitosan–alginate–fucoidan interaction for bone regeneration. Chitosan composite scaffolds with bioceramics, growth factors and stem cells, will have applications in the field of bone tissue engineering. But the road from research and development through regulatory issues to commercialization is not really a joy ride, he pointed out.

Shruthi Eshwar, KLES Institute of Dental Sciences, Bengaluru talked about the uses of chitosan in dentistry. She compared the use of chitosan with the traditional EDTA in smear layer removal and found that 0.2% chitosan is as effective as 17% EDTA. She also compared the use of conventional glass ionomer cement against chitosan-modified glass ionomer cement and found that, though the setting time increased, incorporating chitosan reduces bacterial load in saliva and is, thus, safer for use. She compared low molecular weight chitosan and commercially available denture cleanser on the candidal biofilm formed on dentures and found that chitosan is an effective dental cleanser. Experiments also showed that chitosan, incorporated in dentine hypersensitivity agents, reduces pain perception. Chitosan with concentrated growth promoters also showed promise in alveolar bone regeneration. A pilot study with chitosan gel with concentrated growth promoters from the patients themselves showed that healing after extraction and implants is better with chitosan.

Prajakta Dandekar Jain, Institute of Chemical Technology, Mumbai talked about the protective effect of chitosan nanoparticles on labile therapeutic molecules for enhancing their intracellular delivery. His lab prepared chitosan nanoparticles incorporating therapeutic molecules such as siRNA and amphotericin B, using ionotropic gelation. The nanoparticles were internalized by cells through passive diffusion, irrespective of cell lines. After dissociation of the drugs from the nanoparticles, the cells retained them without any impact on the integrity and viability of the cells. Experiments with small RNAs, that interfere with protein expression, showed that they can be delivered using chitosan nanoparticles to silence specific genes. This opens up a plethora of applications in individualized treatments targeting genes of tuberculosis bacteria, cancers and some genetic diseases.

M. V. Badiger, NCL Pune, situated chitosan in the context of polymer hydrogels in general. Many biopolymers can form 'smart' hydrogels that respond to stimuli – a change in the environment. Chitosan has some of the most important properties for a hydrogel for biomedical applications. Badiger discussed the development of PEG-g-Chitosan as injectable gel for sustained release in his lab. He also discussed the various techniques used for investigating smart hydrogels.

It was clear from the proceedings that, while marine sources of chitin would play the role of raw material for applications in agriculture and environment, chitin–chitosan from fungal sources would be important in medical applications. Positioning itself between glucose and amino acid metabolism, chitin synthesis would thus need the attention of researchers and industrialists seeking to produce high amounts of chitosan of uniform quality. Narayan S. Punekar, IIT Bombay gave important insights into chitin synthesis in *Aspergillus niger*.

Environmental technologies

S. Meenakshi, The Gandhigram Rural Institute, Dindugal discussed the problem of fluorosis and the need to find cost effective methods to remove fluorides from water. She gave an account of strategies using chitosan. Alumina–chitosan composites, hydrotalcite–chitosan composites, polyamidoamine functionalized chitosan beads and chitosan/acrylonitrile – divinylbenzene–vinyl benzyl chloride resin blends were used to remove fluoride. The adsorbents can be regenerated and are thus cost effective, she said.

She also described methods for removing heavy metals using chitosan with combinations of other materials. Besides heavy metals, other common pollutants such as nitrates, phosphates, melamine, dyes, etc. can also be removed using chitosan combined with appropriate materials. She gave examples of strategies that her lab has used with success. She also recounted methods to extract oil from oil spills using chitosan. Besides addressing environmental issues, chitosan can also be used to fight corrosion in industries.

Awards conferred

To honour the researchers who made significant contributions to chitin–chitosan research, the Society has constituted awards. Marine Marshal, a company that led the formation of the Indian Chitin Chitosan Society, supported four Marshal Awards. The society had support from scientists and researchers from the beginning and Mukund Deshpande, from NCL Pune supported a fourth, for young scientists who show their mettle by their publications and patents. This year, no candidate was found suitable for the young scientist award.

The Marshal Award winners for the year showcased their research in the meeting.

P. K. Dutta, Motilal Nehru Institute of Technology, Allahabad who was conferred with the Marshall Award for Researcher in the meeting, recounted his experiences in chitin–chitosan research from 1993.

Nivedhitha Sundaram, Amrita Vishwa Vidyapeetham, Kochi winner of the Marshall Award for Student (Medicine) talked about chitosan based injectable hydrogel as hemostatic agent.

Anu Singh, Motilal Nehru National Institute of Technology, Allahabad Marshall Award winner for Student (Chemistry), gave an account of the properties and application of a chitin–glucan complex from the edible mushroom, *Agaricus bisporus*.

R. V. Kumara Swamy, Maharana Pratap University of Agriculture and Technology, Udaipur, Marshall Award for Student (Agriculture), pointed out an easy way to make crops resistant against fungi. Synthesis of chitosan nanoparticles, using ionic gelation, gave adequate porosity, exposing functional groups to attach copper, he said. The copper chitosan nanoparticles when tested for seed priming, and for foliar spray, gave adequate protection against pathogens and promoted plant growth. The nanoparticles with copper were effective against fungal attacks even at 0.01% and the production cost, at present, is estimated to be a little more than a thousand rupees.

Research, industry, market

Prashant Agrawal, Ira Agrotech and Research Pvt Ltd, Nagpur described his journey from researcher to entrepreneur and spoke about the courage and grit needed to take a research outcome to the market.

S. Sithanantham, Sun Agro Biotech Research Centre, Chennai said that the focus of his lab is control of insect pests using various means. He offered collaboration and support to academic institutions and individuals in areas of interest.

Atul Bedekar, Bioproducts Research and Training Centre, Deogad bemoaned the fact that, though the number of publications on chitosan from India has increased multifold, it has not translated into economic activity. He suggested solutions to overcome the problem and spoke about the potential for research collaboration between academia and industry. He pointed out the large number of colleges in the country and the possibility of good research being done to solve the problems of cottage industries, innovative entrepreneurs, and start ups. He pointed out differences in perspectives between academics and industrialists to stress the need for mutual understanding. He briefly touched upon how new markets for products from chitin are energizing research.

Shashikant Joshi, Everest Biotech, Bengaluru said that, though chitosan has many potential applications, quality is a major concern. For biomedical applications, certification from the Food and Safety Standards Authority of India is needed. He gave examples of dental products that have come into the market. The company has chitosan-based products for agriculture too – for seed coating, for plant growth improvement, for shelf-life enhancement of vegetables and fruits. He offered collaboration for product development, test marketing and pilot-scale production.

Santosh G. Tupe, Greenvention Biotech Pvt Ltd, Pune compared the various sources of chitin and the magnitude of raw material for chitosan production. He pointed out the limitless potential of fungal sources of chitosan. He compared the costs of marine, animal-derived and fungal chitosan to stress the market potential of chitosan from fungal sources. As a reliable and consistent source of good quality chitosan for biomedical purposes, fungal sources are not tapped optimally in India. Though setting up plants exclusively for the purpose makes business sense, biotech industries involved in fungal cultures for other purposes can add value to their business by using waste mycelium for chitosan production. He briefly described his own efforts to produce fungal chitosan at pilot-scale, using NCL technology. The chitosan-based products for agriculture that his company is producing presently were presented.

The panel discussion that followed struggled to get a consensus on the steps to be taken, to align research and industry to market forces. But the excitement and enthusiasm of the audiences, virtually all the who's who in chitin and chitosan research in India, was palpable. Though it has taken 200 years from the discovery of chitin to the multifarious applications, Indian research has come of age and in collaboration with industry, is ready to take on the challenges in the market dominated by Japan, Korea and China.

One of the highlights of the meeting was the release of a special postal envelope for the 7th ICCS-2018 meeting by Col. S. F. H. Rizvi, Postmaster General, Pune and A. K. Nangia, Director, NCL. This, the organizers said, will help reach the message of chitin and chitosan research to the public in a novel manner.

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