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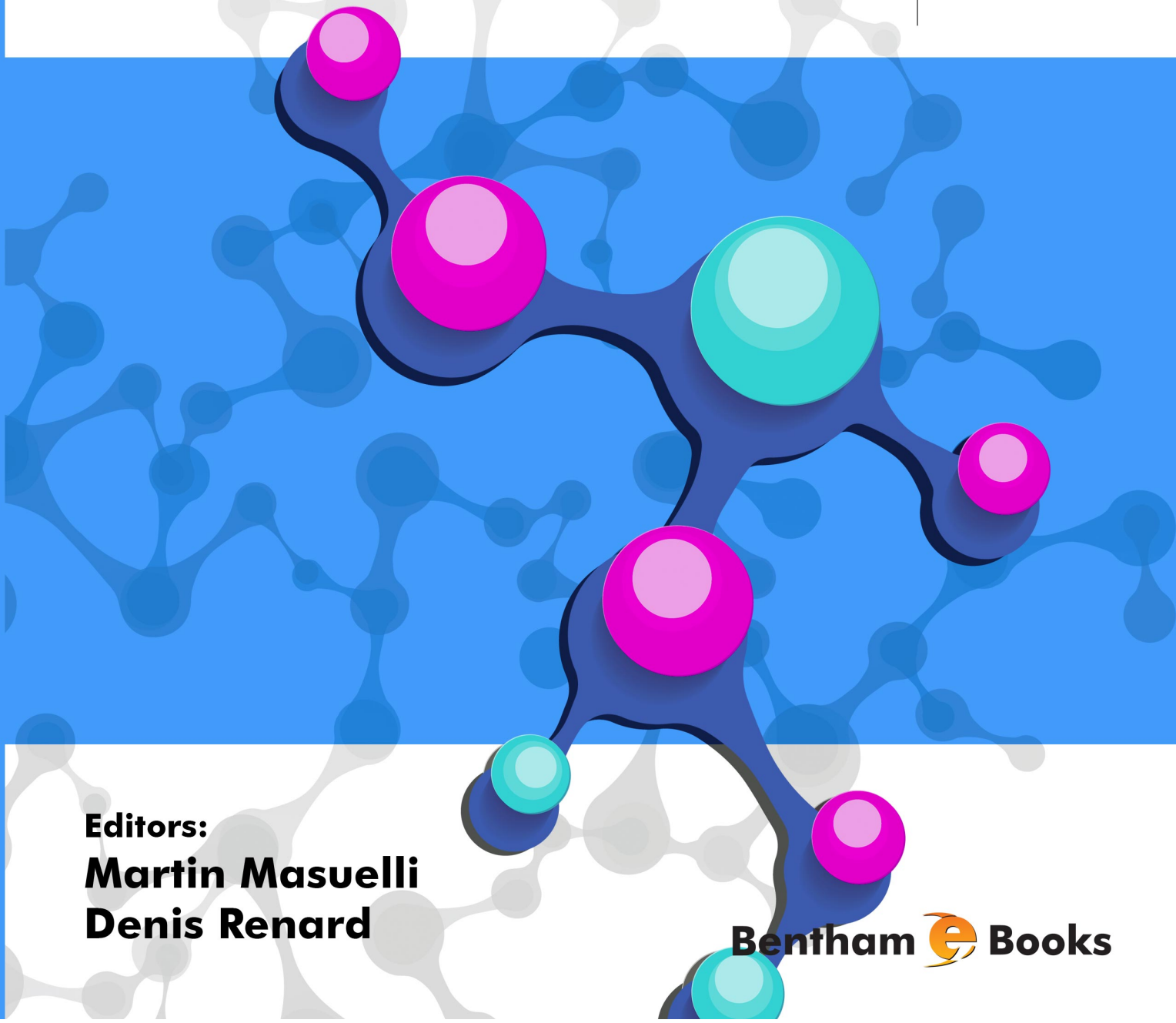
ADVANCES IN PHYSICOCHEMICAL PROPERTIES OF BIOPOLYMERS

Part 2



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Advances in Physicochemical Properties of Biopolymers

(Part 2)

Edited by:

Martin Masuelli & Denis Renard

Instituto de Física Aplicada-CONICET, Universidad Nacional de San Luis, Chacabuco 917, CP 5700, San Luis, Argentina

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PREFACE

The objective of this ebook is to provide to the readers the most recent state-of-the-art on physicochemical properties of biopolymers and their related end-uses applications. Biopolymers are usually described as polymers produced in a natural way by living species. Their molecular backbones are composed of repeating units of saccharide, nucleic acids, or amino acids and sometimes various additional side chains contributing also to their functionalities.

If the largest part of biopolymers is extracted from biomass, such as polysaccharides from cellulose and proteins from collagen, milk or wheat, biopolymers can also be produced from monomers using conventional chemical processes as polylactic acid, or directly in microorganisms or genetically modified organisms, as polyhydroxyalkanoates. The genetic manipulation of microorganisms brings a tremendous potentiality for the biotechnological production of biopolymers with tailored properties quite suitable for high-value medical applications such as tissue engineering and drug delivery.

Biopolymers from renewable sources, on the contrary display structural complexity and natural variability that need to be deeply studied and characterized before probing into the structure-function relationships for further applications. Research on natural polymers has focused on developing more environmentally friendly applications to reduce pollution caused by non-biodegradable material. Historically, biopolymers were mainly used by mankind as food, or for making clothing and furniture. Since the industrial time, fossil fuels such as oil are the greatest source in the development and manufacture of almost every commercial product, such as the plastic, which is currently used at a very large scale. But these fuels are not unlimited resources, and environmental concerns over all aspects of using fossil fuels for production and energy must be taken into account. We must act in a sustainable manner, which means that the resources must be consumed at a rate such that they can be restored by natural cycles of our planet [1].

Therefore, in recent years, the renewable nature of biopolymers leads them to a renaissance and a new considerable interest by industry due to the unique properties, including biodegradability, biocompatibility and nontoxicity, of biopolymers. To fulfil all these different functions, biopolymers must exhibit rather diverse properties. They must very specifically interact with a large variety of different substances, components and materials, and often they must have extraordinarily high affinities to them. Finally, they must have a high strength. Some of these properties are utilized directly or indirectly for various applications. This and the possibility to produce them from renewable resources, as living matter mostly does, make biopolymers interesting candidates to industry [2]. As a consequence of their properties, these biopolymers derived from natural products have found a place of choice in areas as diverse as effluent treatment, papermaking, chemical, food, cosmetic, pharmaceutical, petroleum and textile industries, as well as in analytical chemistry (biosensors) and molecular biology. However, biopolymers have to compete with polymers derived from fossil fuel not only because of their functional properties but also in terms of cost. In this respect, biopolymers are competitive when the price of oil is high and the price of feedstocks, such as starch from corn, is low.1 The continuing development of new and existing biopolymers will enable these materials to help supplement the increasing global demand for biopolymers-based products and to develop new markets with their niche applications.

The most common biopolymers used for industrial applications and thoroughly considered in

this ebook are polysaccharides from plant, algal, microbial and animal origins such as starch, cellulose, lignin, arabinoxylans, sulfated polysaccharides from seaweeds, galactomannans and xyloglucans from Brazilian seeds, chitin and its derivative chitosan. Natural gums such as mesquite, tara and arabic gums are also widely used in food and non-food industry and are discussed in this ebook. Animal and plant proteins such as collagen, gelatin, albumin, dairy proteins and wheat, corn and soy proteins are also considered as sources of proteins for biomedical, microencapsulation and plastic foams applications. Nucleic acids such as DNA and RNA and their related applications in genetic engineering for instance are not considered in this ebook.

This ebook presents a comprehensive review and compile information on biopolymers in 27 chapters covering from isolation and production, properties and applications, modification, and relevant analytical methods to reveal the structure and properties of some biopolymers.

Authors write this ebook from Argentina, France, Mexico, Spain, Iran, Brazil, Egypt, Turkey, Venezuela, India, Russia, Portugal, New Zealand and Malaysia. This ebook has tried to arrange the ebook chapters in a subject order to make it easier for the readers to find what they need. However, the reader can still find information on the same subject in more than one Section.

Section A (Part 1), which includes one chapter, is mainly an introduction to biopolymers. It includes concepts and molecular weight determination.

Section B (Part 1), which includes twelve chapters, refers to some physical chemistry determinations of biopolymers.

Section C (Part 1), which consists of two chapters, deals with studies on hydrodynamic properties of biopolymers.

Section D (Part 1), which consists of one chapter, refers to theoretical models for biopolymers.

Section A (Part 2), which includes four chapters, refers to special cases of polysaccharides separation and purification.

Section B (Part 2), which includes seven chapters, deals with applications of biopolymers/hydrogels in drug delivery systems, biomaterials, biothermoplastics, bio(nano)composites, bionanostructures, biocapsules, bioadsorbents, bioelectrospinning and biopackaging. This section deserves a special attention because it forms a fascinating interdisciplinary area that brings together biology, chemistry, materials science and (nano)-technology.

This ebook is expected to be of help to many graduate and post-graduate students, professors, scientists, pharmacists, engineers and other experts in a variety of disciplines, both academic and industrial, dedicated to the determination of polymers and biopolymers properties. This ebook may not only support research and development, but also be suitable for teaching. The audience will benefit with an excellent review offering advanced knowledge about technical determinations and physicochemical properties of macromolecules, a thorough knowledge of hydrodynamics and different methods of characterization. Readers will find in this ebook a triple deal, including educational, scientific and industrial applications.

The first main objective of this e-book is therefore to highlight the progress in different techniques of molecular weight determinations and physicochemical properties of

biopolymers. The last two decades have seen a number of significant advances in the methodology for evaluating the molecular weight distributions of polydisperse macromolecular systems in solution at the molecular level. These advances have centered on the coupling of chromatographic or membrane based fractionation procedures with multiple detectors on line such as multi-angle laser light scattering, refractive index, UV-Vis absorbance and intrinsic viscosity detection systems. Recent advances in SEC-MALLS (size exclusion chromatography coupled to multi-angle laser light scattering) and FFF-MALLS (field flow fractionation coupled on line to MALLS) applied to complex polymers from renewable resources are therefore presented in this e-book. Beyond molecular charcaterization using HPSEC-A4F-MALLS technique, tremendous efforts were made these last years to elucidate the structural variability and complexity of polysaccharides using matrix-assisted laser-desorption ionization (MALDI) and electrospray ionization (ESI) mass spectrometry coupled or not to nuclear magnetic resonance (NMR) spectroscopy. One chapter of this ebook in section B considers the sequence, interresidue linkage position and substitution pattern of sulfated polysaccharides after enzymatic hydrolyses.

The most widely used method for the dynamic characterization of macromolecules in solution is the capillary viscometry, as it is a simple and economic method. Although in literature there is much information on hydrodynamic measurements from intrinsic viscosity determinations, very few of them evaluate the conformation of different biopolymers. The importance of this type of study lies in the analysis of the polysaccharides or proteins behaviour in industrial processes and product quality control after extraction and purification. These physicochemical studies help to elucidate the chemical structure, macromolecular conformation and the ability biopolymers have to form gels, films, agglomerates, *etc*. A particular attention is paid in this ebook on the intrinsic viscosity determination of proteins and strong synthetic polyelectrolytes for which theoretical models always need to be implemented in order to get reliable dynamic structural informations.

The ebook also focuses on the structural analyses at the mesoscopic scale using mechanical analyses, microscopy, small angle scattering and free volume measurements and different applications related to biopolymers such as biomaterials, microcapsules, biothermoplastics, nanostructured biocomposites, super-absorbents, bioelectrospinning, biopolymers-based dermal and transdermal drug delivery systems, and biopackaging. All these applications using biopolymers aim to provide a means to reduce dependence on fossil fuels, and decrease the environmental impact of non-biodegradable materials. The main challenge to overcome with biopolymers-based materials is the control of biopolymer-biopolymer interactions, a challenge always present and discussed throughout the ebook by authors.

To conclude, the content of this ebook will bring its readers a basic understanding of the physical chemistry of biopolymers, but also the latest findings about new macromolecules recently discovered and published. Theoretical aspects of computational structural description of biopolymers are also thoroughly described. Therefore, this ebook will appeal to different readers as a great source of knowledge about the science of biopolymers.

Biopolymers Audience

- Separation, purification, characterization of biopolymers
- Hydrodynamic, molecular weight, size, shape, conformation
- Macromolecular assembly
- Molecular design and bio-nanotechnology
- Biopolymer processing and degradation

- Experimental and theoretical studies of biopolymer structures
- Three-dimensional structures of biopolymers determined by X-ray, neutrons, NMR
- Interactions and thermodynamics
- Food biocolloids
- Structure and function
- Preparation and characterization of novel biomaterials
- Capsules and microcapsules
- Biocatalysis
- Biopolymers for bioremediation
- Thin films, membranes & packaging

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Denis Renard
Unité de Recherches Biopolymères,
Interactions, Assemblages,
INRA, Rue de la Géraudière,
France
Email: drenard@nantes.inra.fr

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List of Contributors

Alejandra L. Valdez	Laboratorio de Micodiversidad y Micoprospección, PROIMI-CONICET, Tucumán, Argentina Cátedra de Microbiología, Facultad de Bioquímica, Química y Farmacia (UNT), Tucumán, Argentina
Andreia Almeida	i3S – Instituto de Investigação e Inovação em Saúde, Porto, Portugal INEB – Instituto de Engenharia Biomédica, Porto, Portugal IIFACTS – Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde, Gandra, Portugal
Anuradha Walallavita	School of Engineering, Faculty of Science and Engineering, University of Waikato, Hamilton, New Zealand
Athira K. Sunil	Indian Institute of Science, Bangalore – 560012, India
Bruno Sarmento	i3S – Instituto de Investigação e Inovação em Saúde, Porto, Portugal INEB – Instituto de Engenharia Biomédica, Porto, Portugal IIFACTS – Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde, Gandra, Portugal
Carmen L.O. Petkowicz	Biochemistry and Molecular Biology Department, Federal University of Paraná, Curitiba-Paraná, Brazil
Casparus J.R. Verbeek	School of Engineering, Faculty of Science and Engineering, University of Waikato, Hamilton, New Zealand
Chanelle Gavin	School of Engineering, Faculty of Science and Engineering, University of Waikato, Hamilton, New Zealand
Clayton Fernandes de Souza	Chemistry Undergraduate Program, School of Education and Humanities, Pontifical Catholic University of Paraná, Curitiba-PR, Brazil
Entsar I. Rabea	Department of Plant Protection, Faculty of Agriculture, Damanhour University, 22516-Damanhour, Egypt
Jesica Gassmann	Instituto de Física Aplicada-CONICET, Universidad Nacional de San Luis, Chacabuco 917, CP 5700, San Luis, Argentina Policlínico Regional San Luis, San Luis, Argentina
Joana Léa Meira Silveira	Biochemistry and Molecular Biology Department, Federal University of Paraná, Curitiba-Paraná, Brazil
Julia I. Fariña	Laboratorio de Micodiversidad y Micoprospección, PROIMI-CONICET, Tucumán, Argentina Cátedra de Química Biológica, Facultad de Ciencias Exactas y Naturales (UNCa), Catamarca, Argentina
Juan Manuel Galdopórpora	IQUIMEFA-CONICET. Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Química Analítica Instrumental. (1113) Junin 956 Piso 3. Ciudad de Buenos Aires, Argentina
João Pedro Martins	i3S – Instituto de Investigação e Inovação em Saúde, Porto, Portugal INEB – Instituto de Engenharia Biomédica, Porto, Portugal IIFACTS – Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde, Gandra, Portugal

Kelvia Álvarez	Department of Analytical Chemistry, Faculty of Pharmacy, Central University of Venezuela (UCV), PO Box 40109, Caracas 1040-A, Venezuela Institute of Food Science and Technology, Faculty of Sciences, Central University of Venezuela (UCV), PO Box 47097, Caracas 1041-A, Venezuela
Lautaro M. Buffa	National Institute of Industrial Technology, Mar del Plata, Buenos Aires, Argentina
M. Fernanda Horst	South Institute of Chemistry (INQUISUR), National Council of Scientific and Technical Research (CONICET), National University of South (UNS), Bahía Blanca, Buenos Aires, Argentina
Mark C. Lay	School of Engineering, Faculty of Science and Engineering, University of Waikato, Hamilton, New Zealand
Martin Alberto Masuelli	Instituto de Física Aplicada-CONICET, Universidad Nacional de San Luis, Chacabuco 917, CP 5700, San Luis, Argentina
María Victoria Tuttolomondo	IQUIMEFA-CONICET. Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Química Analítica Instrumental. (1113) Junin 956 Piso 3. Ciudad de Buenos Aires, Argentina
Maria Lucia Foglia	IQUIMEFA-CONICET. Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Química Analítica Instrumental. (1113) Junin 956 Piso 3. Ciudad de Buenos Aires, Argentina
Martin Federico Desimone	IQUIMEFA-CONICET. Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Química Analítica Instrumental. (1113) Junin 956 Piso 3. Ciudad de Buenos Aires, Argentina
Mariela Agotegaray	South Institute of Chemistry (INQUISUR), National Council of Scientific and Technical Research (CONICET), National University of South (UNS), Bahía Blanca, Buenos Aires, Argentina
María B. Bonecco	National Institute of Industrial Technology, Mar del Plata, Buenos Aires, Argentina
María Guadalupe García	Instituto de Física Aplicada-CONICET, Universidad Nacional de San Luis, Chacabuco 917, CP 5700, San Luis, Argentina
María G. Martínez Sáenz	National Institute of Industrial Technology, Mar del Plata, Buenos Aires, Argentina
Mohamed E. I. Badawy	Department of Pesticide Chemistry and Technology, Faculty of Agriculture, 21545-El-Shatby, Alexandria University, Alexandria, Egypt
Natalia A. Castillo	Laboratorio de Micodiversidad y Micoprospección, PROIMI-CONICET, Tucumán, Argentina Cátedra de Micología, Facultad de Bioquímica, Química y Farmacia (UNT), Tucumán, Argentina
Neoli Lucyszyn	Chemistry Undergraduate Program, School of Education and Humanities, Pontifical Catholic University of Paraná, Curitiba-PR, Brazil

Tomy J. Gutiérrez Department of Analytical Chemistry, Faculty of Pharmacy, Central University of Venezuela (UCV), PO Box 40109, Caracas 1040-A, Venezuela
Institute of Food Science and Technology, Faculty of Sciences, Central University of Venezuela (UCV), PO Box 47097, Caracas 1041-A, Venezuela
Composite Materials Group (CoMP), Research Institute of Materials Science and Technology (INTEMA), Faculty of Engineering, University of Mar del Plata and National (UNdMP) and National Council of Scientific and Technical Research (CONICET), Colón 10850, Mar del Plata 7600, Buenos Aires, Argentina

SECTION A. SPECIALS CASES: POLYSACCHARIDES

CHAPTER 1

Chitosan and its Modifications as Biologically Active Compounds in Different Applications

Mohamed E. I. Badawy[1,*] and **Entsar I. Rabea**[2]

[1] *Department of Pesticide Chemistry and Technology, Faculty of Agriculture, 21545-El-Shatby, Alexandria University, Alexandria, Egypt.*

[2] *Department of Plant Protection, Faculty of Agriculture, Damanhour University, 22516-Damanhour, Egypt.*

Abstract: A chitosan biopolymer is a reactive functional polymer, which gives possibilities for chemical modifications to generate new properties and functions. Biocompatibility, biodegradability, non-toxicity to mammals, and potential biological activities make this compound with its derivatives advantageous for many applications in different fields, such as agriculture, food and nutrition, biomedicine, pharmaceutics, and biotechnology. In this chapter, we provide collaborative studies of the biological activity of chitosan and its major derivatives in different applications. In addition, the chapter provides the latest technological applications and prospects of products based on chitosan molecule.

Keywords: Biological activity, Chemical modification, Chitosan, Physico-chemical properties, Technological applications.

1. INTRODUCTION

Chitosan is a linear biopolymer which consists of higher than 70% of β-(1-4)- 2-deoxy-β-D-glucopyranose (GlcN) and lower than 30% of β-(1-4)-2-acetamdo-2-deoxy-β-D-glucose (GlcNAc) units linked by β-1,4-glucosidic bonds. It can be obtained through a deacetylation process of purified chitin, a naturally abundant polysaccharide and the supporting material of crustaceans, insects, yeast, and fungi [1 - 4]. The main difference between chitin and chitosan is the degree of *N*-acetylation (DA) [5, 6]. Chitosan is of commercial interest due to its high percentage of nitrogen (~ 6.0-7.0%) compared to synthetically substituted

[*] **Corresponding author Mohamed E. I. Badawy:** Department of Pesticide Chemistry and Technology, Faculty of Agriculture, 21545-El-Shatby, Alexandria University, Alexandria, Egypt; Tel: +2039575269; Fax: +2035972780; E-mail: m_eltaher@yahoo.com

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cellulose (1.25%) that makes it a good chelating agent for metal ions [7]. It has unique characteristics such as biocompatibility, biodegradability, low toxicity to mammals, and possesses reactive functional groups that make it useful in different areas of application related to (i) agriculture (as a biopesticide against plant diseases and pests, seed-coating, postharvest, and controlled release agrochemicals) [8 - 15], (ii) food industry and nutrition (anticholesterolemic dietary products and antimicrobial coatings for fruit and vegetables) [3, 16, 17], (iii) biotechnology (spinning, a dye-binder for textiles, strengthening additive in paper, enzyme and cell immobilization, protein separation, chromatography column matrix, and cosmetic functional ingredients) [18 - 20]; (iv) biomedicine (drug and gene delivery, blood coagulation, wound healing, bone regeneration, immunoadjuvant activity, pharmaceutics, and ophthalmology) [21, 22], and (v) combinations of chitosan with other natural or synthetic polymers (grafting, polyelectrolyte complexation, blends, and coatings) [23]. However, chitosan exhibits a limitation in its solubility and reactivity therefore, many studies have paid attention to modify its chemical structure [12, 24 - 26]. Although several works have been reported to obtain functional derivatives of chitosan by chemical modification, very few attained solubility in general organic solvents [27, 28] and some binary solvent systems [29, 30]. Other several studies modified the structure of chitosan by chemical or enzymatic methods to obtain high biologically active compounds and improve solubility in general organic solvents and aqueous solutions at wide range of the pH [25, 31 - 34]. This chapter covers the literatures dealing with the physicochemical properties of chitosan, the biological activities of chitosan and its derivatives of major importance and applications in different areas.

2. CHITOSAN HISTORY AND ORIGIN

The term “chitosan” (pronounced *Kite-O-San*) was given to deacetylated chitin by Hoppe-Seiler [35]. The name chitin is derived from Greek, meaning “tunic” or “envelope” and it was first discovered in mushrooms in 1811 by Henri Braconnot [36]. In 1823, Odier found the same material in insects and plants and named it chitin [37]. The concept of chitin was further known by Lassaigne, who demonstrated the presence of nitrogen in its structure when purified the coleopteran elytra and *Bombyx mori* exuviae, and then treated the residues with potassium at warm temperature thus obtaining potassium cyanide that confirmed the presence of nitrogen in chitin [38]. In 1859, Rouget treated chitin with strong alkali, which resulted in a substance that could, unlike chitin itself, be dissolved in acidic aqueous solutions, named as chitosan [39].

Therefore, up to date, the chitin and chitosan are mainly produced commercially

from the waste products of crustaceous exoskeletons of aquatic organisms, fungi and insects. Chitin is found in the outer skeleton of insects (20-38%), crabs, and shrimps and lobsters (69-70%). It was also found that chitin is a major structural component of fungal cell wall (5-20%), which is being considered as an alternative source of chitosan and which varies between different species of fungi from 2 to 60% of dry mycelia [40 - 42]. Zygomycetes, especially *Mucor rouxii*, *M. mucedo*, *M. circinelloides*, *Rhizomucor miehei*, *Rhizopus oryzae*, *Phycomyces blakesleeanus*, and *Cunninghamella elegans* have high content of chitin compared to other fungi [43 - 45]. Moreover, Ascomycetes and Basidiomycetes contain significant quantities of chitin and acidic polysaccharides (26-65%) as cell wall components [46]. However, mushrooms including *Lentinus edodes*, *Lycophyllum shimeji*, *Pleurotus sajor-caju*, and *Volvariella volvacea*, contain minor content of chitinous components [47] and fungi of *Oomycetes* and the fission yeasts considered to lack chitin [48]. In contrast, chitosan occurs as a major component in walls of Zygomycetes, but probably is a minor component in many other fungi [49].

The isolation of chitin from shells waste consists of three chemical steps: deproteinization (DP), demineralization (DM), and decolorization (DC); whereby the order of the first two steps is generally considered irrelevant if protein or pigment recovery is not an objective [50]. Purified chitin is then converted to chitosan by hydrolysis of acetamide groups of chitin as a deacetylation process. This process is carried out by two main procedures: the Broussignac process [51], in which chitin is treated with a mixture of solid potassium hydroxide (50% w/w) in 96% ethanol and monoethylene glycol (1:1), and the Kurita process [52], according to which chitin is treated with hot aqueous sodium hydroxide solution (50% w/v). However, the latter is preferred for industrial purposes. In some cases, the deacetylation reaction is carried out in the presence of thiophenol [53, 54] as a scavenger of oxygen or under N_2 atmosphere to prevent chain degradation that invariably occurs due to peeling reaction under strong alkaline conditions. Here, DA took place using a calculated four times excess of NaOH for the total *N*-deacetylation of all amino groups in chitin with reaction time of 1 h at 100°C. These chemical methods are widely used in industrial scale however, the products obtained may suffer some inconsistencies such as protein contamination, inconsistent levels of DA, and hydrolysis of the polymer. In addition, there are some additional problems, such as environmental issues due to the large amount of waste concentrated alkaline solution [43, 55]. Therefore, alternatives to the chemical processes for production of chitin and chitosan are the fermentation and enzymatic techniques which are widely used on an industrial scale [56 - 61]. While chitin remained an unused natural resource for a long time, interest in chitosan polymer and its derivatives has increased in recent years due to their distinctive properties.

3. PHYSICOCHEMICAL CHARACTERIZATIONS OF CHITOSAN

3.1. Chemical Structure

Chitosan is composed of GlcN and remaining of GlcNAc units which indicate high content of free amino groups compared to chitin (Fig. **1**) [6, 62]. The polymer chains appear as linear in structure, which undergoes one full twist, every 10.1 to 10.5 Å along the polymer chain axis. A separate "left" and "right" direction can be occurred to each polymer chain as the presence of each chiral glycosidic unit in the polymer chain, and all units are connected by an oxygen atom that links C-1 of one glycosidic unit to C-4 of a neighboring unit. After heating, chitosan decomposes prior to melting, thus it has no melt point and its crystalline structure is mainly characterized by spectroscopic techniques such as X-ray, UV, FT-IR, and NMR [63 - 69]. The IR spectrum showed main absorption band at 3432 cm^{-1} due to the partially overlapped of the amine and hydroxyl (N-H, O-H, and NH_2) stretching vibrations. Weak absorption bands at 2868 and 2919 cm^{-1} represent a group -CH- and -OH stretching vibration, respectively. The absorption bands at a wave number of 1656 and 1380 cm^{-1} correspond to the C=O stretching of CO and amide group, respectively. The absorption band at wave number of 1597 cm^{-1} was due to the deformation NH of amino groups. The absorption band at 1325 cm^{-1} corresponds to amid III band. While the absorption band at a wave number of 1151, 1092 and 1024 cm^{-1} corresponds to the symmetric stretching of the C-O-C and symmetrical skeletal vibration involved in CO and CN stretching, respectively [70, 71]. In addition, the ^{1}H-NMR spectrum of chitosan is shown in Fig. (**2A**). The peak at δ 2.06-2.13 ppm is assigned to the proton of residual CH_3 in acetyl group. The peak at δ 3.10-3.28 ppm is attributed to H-2 of GlcN residue. The broad multiplet peak at δ 3.53-4.12 ppm is assigned for H-3,4,5,6 of GlcN unit and H-2,3,4,5,6 of GlcNAc unit. The intense band at 5.03-5.17 ppm is related to OH groups and D_2O (solvent). In this region, as observed more clearly from an extended spectrum, some different anomeric protons (H-1 of GlcN and GlcNAc units) are appeared at 4.80-4.91 ppm [72, 73].

Further evidence for confirmation: the chemical structure was obtained from ^{13}C-NMR spectroscopy. Strong and intense peaks for carbon atoms are found at δ 25.95 (NH(CO)$\underline{C}H_3$), 56.37 (C-2), 60.99 (C-6), 70.74 (C-3), 75.44 (C-5), 77.80 (C-4), 98.21 (C-1), and 178.24 ($\underline{C}(O)CH_3$).

CHAPTER 2

Biopolymers of Microbial Origin

Natalia A. Castillo[1,2], Alejandra L. Valdez[1,3] and **Julia I. Fariña[1,4,*]**

[1] *Laboratorio de Micodiversidad y Micoprospección, PROIMI-CONICET, Tucumán, Argentina*

[2] *Cátedra de Micología, Facultad de Bioquímica, Química y Farmacia (UNT), Tucumán, Argentina*

[3] *Cátedra de Microbiología, Facultad de Bioquímica, Química y Farmacia (UNT), Tucumán, Argentina*

[4] *Cátedra de Química Biológica, Facultad de Ciencias Exactas y Naturales (UNCa), Catamarca, Argentina*

Abstract: Synthetic polymers play a significant role in the technological development of humankind and are obtained from petroleum by-products. Because of their physical properties, they can be used for the manufacture of a diversity of products ranging from simple garbage bags or contact lenses up to products for construction. Based on plastic skills such as low price, low weight, resistance to abrasion, impact and corrosion, inertia and versatility, they gradually replaced traditional materials like wood, stone and metal. However, these same advantages nowadays have become their worst drawback, turning them into wastes of difficult disposal and consequently, in a serious environmental problem. Additionally, their multiple usage in daily life has caused a massive increase in consumption and the consequent pollution problems. In this context, biopolymers have emerged as an ideal alternative to the synthetic polymer industry. Biopolymers provide a solution to the origin of the problem as coming from renewable resources, practically all of them being biodegradable, which is not the case for most synthetic polymers. Eco-friendly waste disposal of biopolymers takes advantage of their property of being degraded by soil microbiota, which significantly reduces CO_2 emission as compared to conventional incineration. Therefore, the use of biodegradable biopolymers is also relevant from the point of view of global warming prevention. Based on this rationale, biopolymers based on renewable resources are generating an increasing interest, both in the overall society and particularly, in the plastic industry.

Keywords: Applications, Biopolymers, Downstream processing, Exopolysaccharides, Fermentation, Physicochemical properties, Sustainable production.

[*] **Corresponding author Julia I. Fariña:** Laboratorio de Micodiversidad y Micoprospección, PROIMI-CONICET, Tucumán, Argentina; Tel: +54 381 4344888 #23; Fax: +54 381 4344887; E-mails: jifarina@yahoo.com, jifarina@proimi.org.ar

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INTRODUCTION

Although a great controversy exists concerning the definition and scope of the "biopolymer" term, it may be properly ascribed to all macromolecules produced by biological systems including plants, animals and microorganisms. In a step further, polymers chemically synthesized from biological building blocks (*e.g.* amino acids, sugars or lipids) can also be considered as biopolymers.

In the recent years, polysaccharides of microbial origin have gained special interest, compared to the gums from plants and algae. This can be mainly due to the following advantages:

- Specific biosynthetic processes allow obtaining polysaccharides of controlled purity and molecular weight.
- Production is not subjected to geographical and/or climatic variations as happens with plants and algae. These factors can profoundly affect the quality and quantity of the product obtained and the availability of the natural source.
- The possibility to use regional agro-industrial by-products as raw materials for biopolymer synthesis.
- The potential to change the characteristics of the producing organism in order to increase production, or to produce polysaccharides with a special (tailor-made) property.
- The ability to produce a wide variety of polysaccharides, some with high similarities to those of plant or animal origin, although most with unique and distinctive features.

Microorganisms can synthesize biopolymers of diverse chemical classes, *e.g.* nucleic acids, polyamides (proteins and poly-amino acids), polysaccharides, organic polyoxoesters (such as poly-hydroxyalkanoic acids), polythioesters, inorganic polyesters with polyphosphate, and others. These biopolymers fulfil quite different essential functions for the microorganisms such as conservation and expression of genetic information, catalysis of bioreactions, storage of energy and nutrients, communication with other microorganisms, resistance to hazardous environments and structural stability, among others. It has been widely described that many of these biopolymers, more frequently polysaccharides, also possess biological effects when administered by different routes to animals and/or humans. Based on this feature, these are called 'biological response modifiers' (BRMs). These biopolymers bind to pattern recognition receptors in the host, triggering the immune response. Exogenous BRMs have been reported to have anti-viral, anti-bacterial, anti-fungal, anti-parasitic, and anti-tumor activities. Another functional property of polysaccharides is their ability to alter the rheological properties of water, such as viscosity. Finally, bioplastics constitute a

relevant group within biopolymers, which can be processed using the same technologies that are used with conventional thermoplastic materials such as extrusion, injection or blow. Because of all these diverse and unique properties, biopolymers are used by different industries for a wide spectrum of applications, covering areas such as medicine, pharmacy, agriculture, food, and many others.

Biotechnological production of these polymers is, at present, mostly achieved by fermentation with selected microorganisms in stirred tank bioreactors. Depending on the polymer and the producing microorganism, they can be obtained as extracellular or intracellular compounds. Alternatively, biopolymers can also be produced by enzymatic *in vitro* processes. However, some of these biopolymers still face a number of challenges, such as the optimization of production and recovery processes to reduce production costs, as well as the standardization and strict control of the process in order to obtain products with homogeneous features.

In this chapter, we will review some of these biopolymers of microbial origin that have become of commercial interest, because of their outstanding applications and economic competitiveness.

Biopolymers: Towards a Comprehensive and Appropriate Definition

Nowadays, the long-standing definition of biopolymer as "a molecule of high molecular weight formed by monomers that come from a biological system" is still the most accepted one [1]. Accordingly, bio-based biopolymers may come from plants, animals or microorganisms (Table **1**). Nevertheless, there is some discrepancy concerning this definition, and other authors also consider those polymers as biopolymers which come from fossil fuels and exhibit the condition of being biodegradable. Regarding the first definition, another common misunderstanding is to believe that all the biopolymers are biodegradable. Although this is a frequent feature among biopolymers, it is not an absolute fact (Table **1**); the non-degradability ratio among bio-based biopolymers has increased [2].

According to IUPAC recommendations, when biopolymers are man-made, they should be named synthetic biopolymers (Table **1**), to make a distinction with true biopolymers [3].With respect to the natural sources, biopolymers can be extracted from plants and animals, but their industrial production from microbial sources has currently become more relevant due to the advantages of these processes and their high commercial interest [4, 5].

CHAPTER 3

Analysis of Biopolymers from Brazilian Seeds

Joana Léa Meira Silveira[1,*], **Neoli Lucyszyn**[2], **Clayton Fernandes de Souza**[2] and **Carmen Lúcia de Oliveira Petkowicz**[1]

[1] *Biochemistry and Molecular Biology Department, Federal University of Paraná, Curitiba-Paraná, Brazil*

[2] *Chemistry Undergraduate Program, School of Education and Humanities, Pontifical Catholic University of Paraná, Curitiba-PR, Brazil*

Abstract: Polysaccharides, which can be obtained from a variety of different sources, have important industrial applications owing to their properties in solution. Considering the wide variety of plant species found in Brazilian flora, the achievement of these biopolymers from plant seeds is highly promising. In view of this potential, various research groups from Brazil have been investigating several plant species as a source of these natural polymers. These studies include structural elucidation, modification, and industrial application using several approaches. Therefore, the aim of this chapter is to review the results from studies of native Brazilian sources of polysaccharides, especially galactomannans and xyloglucans, to inspire commercial exploitation of these unconventional sources.

Keywords: Brazilian seeds, Carbohydrate, Galactomannan, Hemicellulose, HPSEC-MALLS, Hydrocolloids, Interaction, Leguminosae, Macromolecular analysis, NMR, Nonstarch polysaccharides, Physicochemical analysis, Polysaccharide derivatives, Polysaccharide interaction, Rheology, Structural analysis, Thickener agent, Vegetal polysaccharide, Viscosity, Xyloglucan.

INTRODUCTION

In the seeds of higher plants, polysaccharides have structural and/or storage functions. Of polysaccharides with storage functions, starch is mostly studied and its economically important. However, not all plant seeds have starch as the main storage polysaccharide, and these "nonstarch" polysaccharides include hemicelluloses, amyloids, and mucilage [1]. Such polysaccharides are stored outside the plasmalemma and are therefore called reserve celluloses, and are

* **Corresponding author Joana Léa Meira Silveira:** Biochemistry and Molecular Biology Department, Federal University of Paraná, Curitiba-PR, Brazil; E-mail: jlms12@yahoo.com

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found mainly in seeds, roots, rhizomes, tubers, and bulbs [2]. Studies on the physiology of plants have revealed that the seed germination process is dependent on the type and monosaccharide content of the polysaccharides present in the seeds [3]. To begin seed germination, these polysaccharides are degraded by enzymatic hydrolysis. Moreover, this process and all biosynthesis mechanisms are dependent on the sequence of the oligosaccharides [4].

In general, to explore these polysaccharides, the biopolymers in the plant are first isolated, followed by elucidation of their structures and evaluation of their biochemistry and physiological properties. One advantage of these storage polysaccharides compared with structural polysaccharides in seed cell walls is an easier extraction process [5, 6]. Polysaccharides with good water affinity can be isolated from seeds by aqueous extraction. A simple extraction procedure includes enzymatic inactivation, exhaustive water extraction (cold and/or hot), and precipitation using organic solvents [7 - 9]. In addition, some authors [10, 11] have used new methods to improve the polysaccharide isolation process, including pressurized liquid extraction, ultrasonic-assisted extraction, and microwave-assisted extraction.

The goal of these extraction procedures is to obtain the water-soluble polysaccharides (called hydrocolloids), free of contaminants and in high yields [7, 12 - 18]. Hydrocolloids form colloidal systems in water usually exhibit highly viscous behavior, forming gels consisting of three-dimensional networks of polysaccharide chains [18 - 22]. The absence of toxicity of these biopolymers favors their use in the textile, pharmaceutical, biomedical, cosmetics, and food industries [5, 6, 18, 23 - 25].

The properties of this biopolymers class in solution are dependent on the polysaccharide structure and to determine the primary structures of the polymers, it is necessary to identify the carbohydrate components, their monosaccharide ratios, the manner in which they are linked, and their monosaccharide sequence [26 - 35].

These biopolymers are normally subjected to several methodologies and techniques for structural and physicochemical elucidation, including chromatography high-performance liquid chromatography (HPLC) and gas-liquid chromatography (GLC), mass spectrometry (MS), and spectroscopic methods [nuclear magnetic resonance (NMR), UV-Vis, and Fourier transformed infrared spectroscopy (FTIR)] to gain information about molar mass, dispersity, internal linkages, composition, and monosaccharide sequences [30, 33 - 38].

Due to the complexity of polysaccharides, chromatographic methods are the most commonly used techniques to obtain physicochemical parameters. Purified

polysaccharides can be analyzed by multi-angle laser light scattering (MALLS) coupled to high-performance size-exclusion chromatography with refractive index detection (HPSEC-RI-MALLS), which permits the determination of molar mass, the presence of aggregates, and the homogeneity of the polymers. This technique has been considered an absolute method for characterization of high molar mass polymers [39 - 51].

Light scattering measurements are one of the most important techniques for characterizing dilute polymer solutions [40 - 42]. Static and dynamic laser light scattering (SLS and DLS, respectively) methods, which measure the patterns of light scattered from a polymer in solution, can be used to evaluate the hydrodynamic dimensions of a polymer, which varies with its structure, and thus provide qualitative and quantitative information on the architecture of macromolecules [35, 46, 49, 51].

GLC and HPLC are commonly used for the determination of mono- and oligosaccharides with high efficiencies in separation, quantification, and speed of analysis. Normally, the monosaccharide composition is obtained by acid hydrolysis of the polysaccharides and subsequent evaluation by HPLC. For GLC analysis, the polysaccharides are converted into volatile derivatives, such as methylated alditol acetates, which can be used to predict the presence and position of branches in polysaccharide structures [35, 47, 49].

In addition, 1D and 2D NMR techniques can be employed to obtain information on the pattern of branching, monosaccharide ratios, anomeric configuration (α and β), and oligosaccharide sequences in the complex structures of polysaccharides. The advantage of this technique is that it is nondestructive and requires only a small amount of sample [35, 48].

Many studies have revealed that almost all polysaccharides obtained in high concentrations from leguminous seeds are xyloglucans (XGs) and galactomannans (GMs). The diversity of sources allows for a high diversity of side chains on the XG and GM backbone, resulting in variable physicochemical properties [12 - 14, 20, 46, 52 - 54].

Different research groups from Brazil have evaluated the characteristics, including structural and physicochemical properties of XGs and GMs from several native Brazilian sources. Considering the potential of these biopolymers compared with commercial sources, we present the structural characterization, chemical modification, and possible industrial applications of these polysaccharides.

CHAPTER 4

Chitosan, From Residue to Industry

María B. Bonecco*, **María G. Martínez Sáenz** and **Lautaro M. Buffa**

National Institute of Industrial Technology, Mar del Plata, Buenos Aires, Argentina

Abstract: Industrial activity associated with processing of crustaceans generates large quantities of waste. The main residue corresponds to its exoskeletons constituted primarily of chitin. Through a modification on its structure, chitosan is derived, the only polycationic polymer in nature. This work aims to review a wide variety of aspects concerning chitosan. First it studies the chitosan properties: biodegradability, biocompatibility and non-toxicity. Its high density of positive charges makes it a versatile material, being able to be used in practically all the industrial fields. Agriculture, biotechnology, food, wastewater treatment and medicine are the main examples. Then it compares different chitin and chitosan obtaining methods being mostly used chemical treatments. The biological methods are presented as an alternative. Several techniques are necessary for chitosan´s characterization. Molecular weight and deacetylation degree are the most important characteristics which define its potential applications. This chapter analyzes different methods according to the necessity of each situation. An examination on the regulatory status and the global market of this biopolymer is made. A report in 2010 projected its market in 21.4 thousand tons by 2015, placing Japan as the biggest contributor. The main industries are placed in Asia and US. In Latin America, there are plants in Brazil, Chile and México. In Argentina, despite the crustaceans fishing industry continuously growing, there is no industrial chitosan production. This review concludes with a description of the work that is being carried out at the National Institute of Industrial Technology of Argentina in this regard.

Keywords: Antimicrobial, Biocompatibility, Biodegradability, Biopolymer, Characterization, Chitin, Chitosan, Deacetylation degree, Fishing industry, Market, Molecular weight, Non-toxicity, Obtaining process, Polycation, Regulations, Shrimp, Waste.

INTRODUCTION

In the last century, economic and industrial growth related to the development of societies has led to a corresponding increase in the volume of waste generated worldwide. As a consequence, its final destination has become one of the major

* **Corresponding author María B. Bonecco:** National Institute of Industrial Technology, Mar del Plata, Buenos Aires, Argentina; Tel/Fax: 54-0223-4802801; E-mail: bbonec@inti.gob.ar

problems that communities have to confront. Waste management, both locally and globally, is vital for the preservation of the environment and public health. Industrial activity associated with the processing of fishery products, crustaceans in particular (*i.e.* shrimp and crab), generates large quantities of waste. Despite being an excellent raw material, in many regions, it has become a pollutant of high environmental impact (Nuñez Rico, 2010 [1]). The problem is not only the volume of accumulated waste, but also its slow rate of degradation. This has led to the establishment of different lines of research which seek to find possible solutions to the environmental problem in addition to the potential economic exploitation of the resource.

One of the main residues which results from processing crustaceans corresponds to their exoskeletons; rigid structures that give support and protection to those animals. The most important structural component is chitin, which is the most abundant polysaccharide in nature after cellulose. Chitin does not have a wide range of uses in the chemical industry due to its insolubility in water, organic solvents and dilute acids. However, its importance resides in the possibility of changing its structure in order to obtain another polysaccharide, chitosan (Khor & Lim, 2003 [2]).

Chitosan is the only polycationic polymer in nature. The high density of its positive charges is responsible for its unique physical, biological and physiological properties. Chitosan in its solid state is a semi-crystalline polymer. It is biodegradable, biocompatible and non-toxic (Peh *et al.*, 2000 [3]). It is also well known for its antimicrobial and filmogenic properties (Skjak-Braek *et al.*, 1989 [4]). The molecular characteristics of chitosan make it an incredibly versatile material, allowing it to be used in several ways, such as solutions, films, hydrogels, fibers, porous structure scaffolds, pastes, sheets, tablets, nanoparticles, microspheres, among others. This versatility makes it suitable for being used in practically every industrial field with food and beverage, biotechnology, agriculture, wastewater treatment, medicine, pharmaceutical and textiles being the most important examples.

The discovery of chitosan dates back to 1859, with the studies of Professor C. Rouget, who reported deacetylated chitin forms. In 1894, researchers Hoppe and Seyler called it chitosan (Lárez Velásquez, 2006 [5]). Between the 30's and 40's, these polymers attracted a considerable amount of attention, registering a total of 50 patents. Chitosan has been considered the biopolymer of the 21st century because its study and resulting applications have grown exponentially during the last decades (Nuñez Rico, 2010 [1]). It is important to emphasize the growing activity developed around chitin and chitosan, which has resulted in the organization of congresses, conferences and symposiums dedicated exclusively to

them. Their importance as polymeric materials was first recognized internationally during the 70s, when the book "Chitin" was published by Muzzarelli (1974 [6]) and the First International Conference of Chitin and Chitosan was held in 1978. Latin America has been also working with chitosan. In 2000, the First Latin American Symposium of Chitin and Chitosan was held in Havana (Cuba) during the XI Latin American Symposium on Polymers (Lárez Velásquez, 2003 [7]).

The global importance of chitosan and its industry has led to the authoring of this review, about its production, properties and characterization, as well as its main uses and regulations.

RAW MATERIAL

The raw material used in the production of chitosan are the exoskeletons of shellfish which come from the fish processing industry. The exoskeleton is a rigid non-cellular structure secreted by the epidermal cells of such animals, which are generally divided into three regions: head, thorax and abdomen. The exoskeleton is composed of chitin, proteins, minerals and pigments, where proportions vary depending on the species of crustacean, the backbone region, nutritional status and reproductive cycle of the animal from which they come from. Older specimens have a lot more calcified exoskeletons with a lower proportion of chitin (Tharanathan & Kittur, 2003 [8]). In general, the component proportions are: chitin (15%-40%), protein (20%-40%) and $CaCO_3$ (20%-50%). Carotenoid pigments such as lutein, astaxanthin and β-carotein are found in small quantities along with other mineral elements (Colina *et al.*, 2014 [9]).

In industrial processing, chitin is recovered from crustacean's exoskeletons by alkaline extraction to solubilize proteins followed by acid treatment to dissolve the $CaCO_3$, or *vice versa*. This treatment must be adapted to each chitin source due to the differences in the ultrastructure of the initial raw material (Rinaudo, 2006 [10]). As it will be seen below, this variability constitutes an obstacle to standardize a method for obtaining chitosan, so it is necessary to have different methodologies for the characterization of the obtained product. Table **1** shows the percentages of the major components present in some species of crustaceans.

Table 1. Proximate chemical composition of crustacean's exoskeletons by species (% v/v) on dry basis.

Origin of raw material	Protein	Chitin	Ash content	Lipid
Callinectes sapidus (Crab)[1]	25.1	13.5	58.6	2.1
Chionoecetes opilio (Crab)[1]	29.2	26.6	40.6	1.3
Paralithodes camtschaticus (Crab)[1]	22.0	31.0	46.0	1.0

SECTION B. APPLICATIONS

CHAPTER 5

Amphiphilic Chitosan-Polymer Derivatives and Albumin-Based Formulations for Drug Delivery Applications

João Pedro Martins, Andreia Almeida and **Bruno Sarmento***

i3S – Instituto de Investigação e Inovação em Saúde, Porto, Portugal

INEB – Instituto de Engenharia Biomédica, Porto, Portugal

IIFACTS – Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde, Gandra, Portugal

Abstract: The use of biomaterials as drug carriers is being extensively investigated as a drug delivery strategy in pharmaceutical research. However, the selection of materials for such systems is rather complicated. Lately, the advent of biomaterials brought the use of polymers as drug vehicles into the spotlight.

Chitosan and albumin are two of the most promising biomaterials for the development of drug delivery systems. These are widely available in nature and can be used as scaffolds of unprecedented novel structure, presenting high biocompatibility and biodegradability and reduced toxicity. Moreover, these molecules promote optimized pharmacokinetics for targeted drug delivery and controlled release, and avoid accumulation or undesired side effects in healthy tissues

Chitosan is a linear polysaccharide obtained from chitin and, when used as drug carrier, demonstrates to improve drugs pharmacokinetic profiles when comparing with the drug alone.

Albumin is the most abundant protein in blood plasma, which has been receiving renewed interest in the development of drug delivery systems. Importantly, albumin is already an "off-the-shelf" product.

Advances in the study of engineered biomaterials represent a step forward in the exploitation, development and commercialization of new therapies for old, poorly served medical needs.

Keywords: Albumin, Biomaterials, Biopolymers, Chitosan, Drug delivery.

* **Corresponding author Bruno Sarmento:** i3S – Instituto de Investigação e Inovação em Saúde, Rua Alfredo Allen, 208, 4200-393 Porto, Portugal; E-mail: bruno.sarmento@ineb.up.pt

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INTRODUCTION

The necessity of novel therapies for poorly served medical conditions is triggering the development and approval of new and enhanced drugs. Indeed, sub-optimal pharmacodynamics of many small molecules, peptides and proteins has been driving the development of innovative drug formulations, drug delivery technologies and medical devices [1]. The routes behind this strategy foresee the establishment of advanced formulations, characterized by high biocompatibility and biodegradability, reduced toxicity and optimized pharmacokinetics for targeted drug delivery and controlled release, avoiding accumulation or undesired side effects in healthy tissues [1].

The use of biomaterials as drug carriers is being extensively investigated as a drug delivery strategy in pharmaceutical research. In general, the main functions of a carrier include the protection of the drug from degradation, the enhancement of both drug release profile and absorption (by facilitating diffusion through epithelium) along with distribution and elimination [2]. To complement this research, carrier properties, such as chemistry, surface and functionalization can be precisely tuned to promote a better drug release pattern and biodistribution [2].

Selection of materials for such systems is rather complicated, but lately polymers are being widely used as biomaterials for drug transport.

Chitosan and albumin are two of the most promising biomaterials for the development of drug delivery systems. These are widely available in nature and can be used as scaffolds of unprecedented novel structure [3].

Chitosan is a polysaccharide, structurally similar to cellulose and has the desired properties for safe use as a pharmaceutical excipient [4]. Albumin is a natural polymer, physically and chemically very robust and stable, non-antigenic and metabolizable [1]. Importantly, albumin has an excellent binding capacity to various drugs [5]. The characteristics of these two biopolymers make them very suitable materials for developing drug delivery vehicles.

Advances in this field include the development of chitosan- and albumin-based platforms for drug delivery, aiming toward the treatment of high incident pathological states, as cancer or diabetes. Furthermore, approved therapies with superior therapeutic efficacy are being achieved, and indicate that, in the near future, chitosan- and albumin-based drug delivery systems will have an increased commercial status.

1. CHITOSAN AND AMPHIPHILIC DERIVATIVES

Polysaccharides are widely distributed in nature and, among them, chitosan is receiving increasing attention. Chitosan is a linear polysaccharide prepared from chitin, which is the second most abundant natural polymer in the world, after cellulose [6, 7]. Chitin can be found in the exoskeleton of insects, crustaceans and some cell wall of fungi. Chitosan, derivative of chitin can be obtained by alkaline deacetylation or enzymatic hydrolysis [7]. The reaction product is chitosan only when the average degree of acetylation (DA), is equal to or less than 50%. The DA is used to characterize the average content of *N*-acetyl-D-glucosamine units (acetylated unit) while degree of deacetylation (DD) is the number of D-glucosamine units (deacetylated unit) [6, 8]. Chitosan is constituted by *N*-acetyl-D-glucosamine and of D-glucosamine units linked by glycosidic linkages β-(1,4) (Fig. **1**) [6, 7, 9, 10].

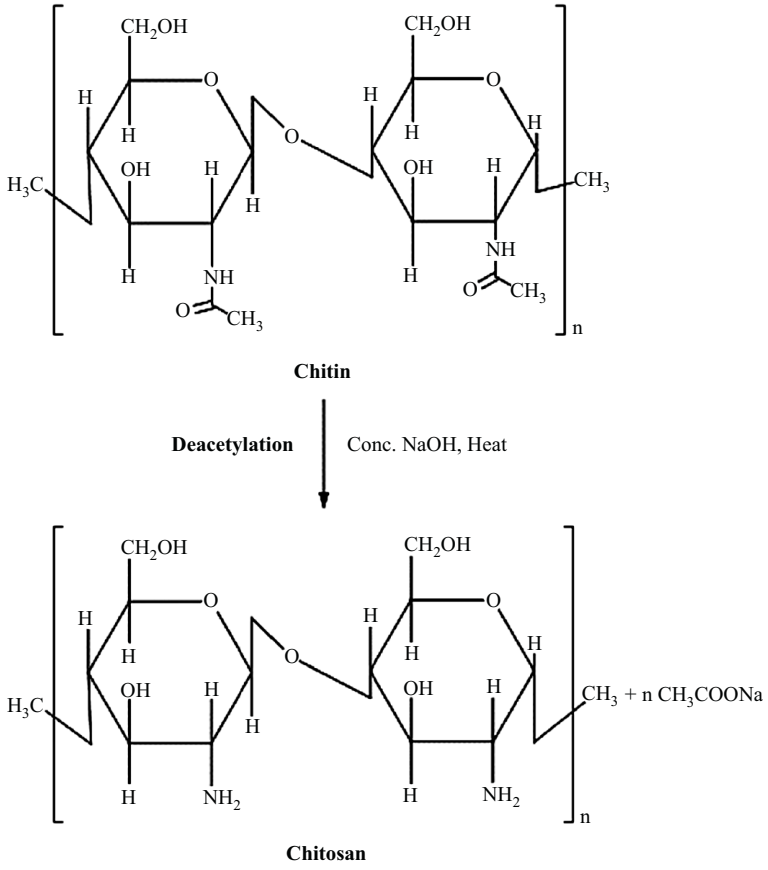

Fig. (1). Chitin deacetylation to chitosan.

CHAPTER 6

Biopolymers as Microencapsulation Materials in the Food Industry

Tomy J. Gutiérrez[1,2,*] and **Kelvia Álvarez**[1,2]

[1] *Department of Analytical Chemistry, Faculty of Pharmacy, Central University of Venezuela (UCV), PO Box 40109, Caracas 1040-A, Venezuela*

[2] *Institute of Food Science and Technology, Faculty of Sciences, Central University of Venezuela (UCV), PO Box 47097, Caracas 1041-A, Venezuela*

Abstract: Microencapsulation is a technology that physically wraps sensitive ingredients in a protective matrix. This may be required for several reasons, such as: 1) to contain aromatic compounds that can be rapidly evaporated or aromas that may be lost during storage, 2) to avoid undesirable interactions between the matrix and the aromas, 3) to minimize interactions between volatile compounds (*flavor*/*flavor*), 4) to protect substances against oxidative reactions, 5) to control and prolong the release of probiotics, drugs and/or *flavor*. Materials that have been microencapsulated include: enzymes, probiotics and microorganisms, acids, bases, oils, vitamins, antioxidants, salts, gases, pharmacologically active peptides and amino acids, flavorings and colorings. Several materials have been employed as microencapsulants, such as: gums (carrageenan, alginate and gum arabic), carbohydrates (starch, maltodextrin, β-cyclodextrin and chitosan), celluloses (cellulose acetate phthalate) and proteins (gelatin and dairy protein isolates). In this chapter we discuss some of the variables, such as the concentrations of the reactants used, that affect the formation of microencapsulation materials.

Keywords: Biopolymers, Food, Microencapsulation.

INTRODUCTION

Microencapsulation is a technology that provides a physical wrapping for ingredients considered as sensitive, in order to protect them from adverse reactions, nutritional deterioration and even the loss of volatile compounds, as a consequence of moisture, heat, or other extreme conditions [1]. This technique has proved to be extremely promising for the development of new products and processes [2].

[*] **Corresponding author Tomy J. Gutiérrez:** Institute of Food Science and Technology, Faculty of Sciences, Central University of Venezuela, PO Box 47097, Caracas 1041-A, Venezuela; Tel: +58 212 7534403; Fax: +58 212 7533971; E-mails: tomy.gutierrez@ciens.ucv.ve, tomy_gutierrez@yahoo.es

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Biopolymers are widely used in the food industry to solve formulation problems that may arise from the chemical or physical instability of the active ingredient, or incompatibility between the active ingredient and the matrix. They may also be used for the controlled release of a compound, or to improve the bioavailability of a particular nutrient [3]. Furthermore, encapsulation is often required to: 1) immobilize cells and enzymes, 2) protect against degradation processes (O_2 and light), 3) extend shelf life and protect the nutritional value of food, 4) prevent the degradation of compounds with high nutritional value, thus maintaining their bioavailability and 5) mask flavors and odors.

The materials most often used for the microencapsulation of food are: gums (carrageenan, alginate and gum arabic), carbohydrates (starch, maltodextrin, β-cyclodextrin and chitosan), cellulose (cellulose acetate phthalate) and protein (gelatin and dairy protein isolates).

Some of the substances that have been microencapsulated for their application in the food industry are: enzymes, probiotics and microorganisms, acids, bases, oils, vitamins, antioxidants, salts, gases, pharmacologically active peptides and amino acids, flavorings and colorings.

Techniques for encapsulation include: spray drying, extrusion, coacervation, ionic gelation, entrapment with liposomes, freeze-drying, co-crystallization and emulsion, and sonochemistry, amongst others. However, the choice of encapsulation method depends on the required average particle size, the physical properties of the encapsulant and the substance to be encapsulated, how the encapsulated material is to be utilized, the desired release mechanism and the cost. The microencapsulation method used should also be quick to apply and versatile.

As already mentioned briefly, selection of the microencapsulation method is based on the physico-chemical characteristics of the polymer and the material to be encapsulated, taking into account the following factors [4, 5]:

- The yield should be high for the desired microparticle size.
- Encapsulation efficiency must be high.
- Reproducibility between batches must be within precise limits.
- The microspheres should not form clusters, and their form should be maintained.
- The environmental impact caused by manufacturing conditions and materials should be minimized.

In addition, the internal morphology of the particle must be controlled as it determines how the active ingredients are released. Particle morphology is heavily

dependent on the microencapsulation method and protocol chosen.

A crucial step in microparticle production is the generation of the drops. This determines the size distribution and structure of the particles, thus affecting their usefulness for each application. The methods currently used for drop preparation are classified into three groups based on their productivity and capacity to control drop size: 1) high productivity and low control of drop size, 2) low productivity and high control of drop size, and 3) high productivity and high control of drop size.

Finally, changes in pH, concentrations of reactants and the temperature of the system also influence on the stability and release mechanism of the encapsulated material.

In this chapter, we discuss basic and general aspects of microencapsulation as an innovative technique for the food industry.

Microencapsulation

Although the term microencapsulation is relatively old, its application is relatively recent. The earliest references describing a microencapsulation process (gelatin microspheres prepared by coacervation) date from 1931. The use of this technology has increased in recent decades due to the interest in the development of biodegradable micro- and nanoparticles.

Microencapsulation is a technology that allows the physical wrapping of sensitive ingredients in a protective matrix (a "wall type" material) in order to protect them from adverse reactions, nutritional deterioration and even the loss of volatile compounds [6] as a consequence of moisture, heat, or other extreme conditions. Microencapsulation thus enables the development or improvement of the stability of the ingredients, as well as maintaining the product viability [1]. This technique has proved to be extremely promising for the creation of new products and processes [2]. Within the food industry, microencapsulation is typically used to resolve formulation problems that may arise from the chemical or physical instability of the active ingredient, or incompatibility between the active ingredient and the matrix. They may also be used for the controlled release of the compound, or the bioavailability of a particular nutrient [3].

From a technological point of view, microencapsulation is defined as the process of coating a product with molecules, solid particles or liquid globules, derived from different kinds of materials, to produce micron-sized particles. There are three types of particles resulting from this technological process: "microparticles", "microcapsules" or "microspheres", which differ from each other in their

CHAPTER 7

Gels in Biomedical Applications: An Overview on Wound Healing and Tissue Engineering

M. Fernanda Horst* and **Mariela Agotegaray**

South Institute of Chemistry (INQUISUR), National Council of Scientific and Technical Research (CONICET), National University of South (UNS), Bahía Blanca, Buenos Aires, Argentina

Abstract: Biopolymer hydrogels present diverse applications in medicine due to their biocompatibility, biodegradability and low immunogenicity. The specific features related to swelling, holding a large amount of water while maintaining the structure, in addition to the ease of taking different shapes, make them the selected biomaterials as component for diverse bio-applications: contact lenses, injectable or implantable drug delivery devices and as platforms intended for wound healing and tissue engineering. In general, the restriction associated to the use of hydrogels in biomedicine lies in the poor mechanical properties associated to the natural biopolymers. This problem can be solved by the use of other materials during the synthesis procedure. In this chapter, general synthesis methodologies and latest innovations in terms of gelatin, collagen and hyaluronic acid hydrogels for wound care and tissue engineering are reviewed. The selection of the biopolymers is based on their suitable features for biomedical applications. The focusing of specific clinical challenges for wound healing and tissue engineering can prove to be beneficial for rapid development in science and marketing. This implies considering the current increasing market associated to hydrogel employment for wound care and treatment as well as the requirement to develop concise clinical hydrogels implementation on the replacement of diverse tissue and organs.

Keywords: Biopolymers, Collagen, Gelatin, Gels, Hyaluronic acid, Synthesis and characterization, Tissue Engineering, Wound healing.

INTRODUCTION

Natural polymers have long been studied and widely used in the last decades in many applications issues such as food and pharmaceutics industries as well as in medicine field. In the recent years, they have become promissory materials for

* **Corresponding author M. Fernanda Horst:** South Institute of Chemistry, National University of South (INQUISUR-CONICET-UNS), Bahía Blanca, Buenos Aires, Argentina; Tel: (54) 2914595101; Int: 3556; Fax: (54) 2914595460; E-mail: mfhorst@uns.edu.ar

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biomedical applications owing to their biodegradability, biocompatibility, non-toxicity and specific therapeutic activities [1]. Biopolymers contain different functional groups, such as hydroxyl, amino, carboxylic acid, aldehydes, and others which make them ideal for conjugation. The study of biopolymers-based hydrogels and the possibility of their employment in living tissues begun analyzed in the early 1960s by Wichterle and Lim who proposed the design of new biomaterials for ophthalmology [2].

In general terms, gels consist of a solid three-dimensional network that extends the volume of a liquid medium and entangles through surface tension effects. The gels could contain crystallites or other junctions that remain intact within the extending fluid. For all practical purposes, any fluid can be used as an extender including water, oil and air. In the case when the extender is water the gels are usually known as *hydrogels* and when the extender is air, they are named as *aerogels.*

At that point, hydrogels consist of three-dimensional, hydrophilic, polymeric networks with soft and rubbery consistence being thus similar to living tissues. They are capable to retain large amounts of water or biological fluids, so they can absorb from 20% up to thousands of times their dry weight in water. They may be chemically stable or could degrade and eventually disintegrate and dissolve [3, 4].

Softness and rapid diffusion ability of molecules make them useful for a wide variety of bio-applications (Fig. **1**). Soft contact lenses made of hydrogels became commercially successful, since these polymeric systems allow controlling all necessary features through the molecular design. Gels composed by diverse polymers such as poly(2-hydroxyethyl methacrylate) and its copolymers with methacrylic acid; 1-vinyl-2-pyrrolidone and its copolymers with 2-hydroxyethyl methacrylate and alkyl methacrylates; and poly(vinyl alcohol) and its derivatives are considered unique materials for the development of contact lenses [5].

Hydrogels are extensively employed in the development of drug delivery systems (DDS) due to their common properties related to swelling and holding a large amount of water while maintaining the structure. Moreover, they present a controllable diffusion rate through the induction of changes in the crosslink density [6]. These features make them suitable for drug protection from hostile environments in the body, such as the presence of enzymes and low pH in stomach, and also to control drug release [7]. Hydrogels present different devices forms as DDS: injectable or implantable depots and nanogels. The latest trend is the development of smart-hydrogels: environmentally sensitive gels which respond to pH or temperature changes in the body and self-regulated DDS which respond to specific stimuli, for instance, self-regulated insulin delivery hydrogels

sense glucose concentration to control swelling to release the hormone [8].

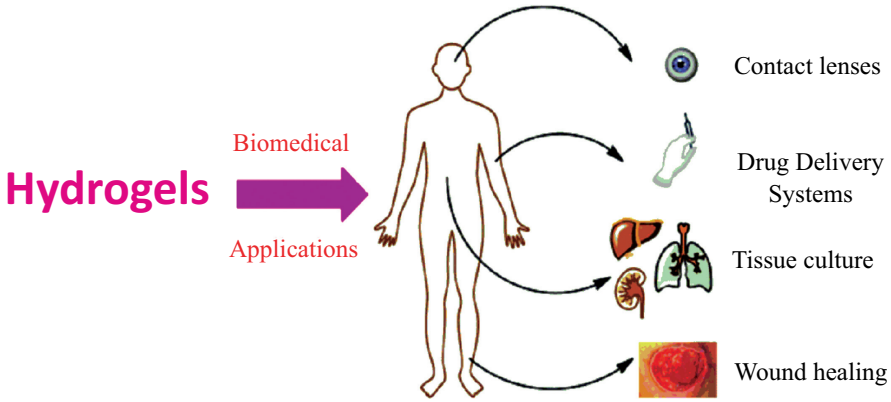

Fig. (1). General biomedical applications of hydrogels.

There are two areas of interest in biomedicine where hydrogels application is of greatest importance: wound healing and tissue engineering. The skin lesions induce a cascade of processes leading to re-epithelialization and re-establishment of skin function. If this process misleads, it becomes a stagnation of the repair process causing a chronic wound. Therefore, hydrogels arise as a malleable alternative for wound healing. In tissue engineering insights, hydrogels use attempts to mimic extracellular matrix and lead to the replacements of organs or implantations.

Wide and diverse biopolymers have been used to fabricate hydrogels for biomedical applications. This chapter focuses on the latest trends and innovations of collagen, gelatin and hyaluronic acid hydrogels for wound healing and tissue engineering.

HYDROGELS: SYNTHESIS AND PHYSICOCHEMICAL PROPERTIES

Collagen, gelatin and hyaluronic acid among others, are protein and polysaccharides widely used and investigated to obtain hydrogels for wound healing and tissue engineering applications. In this section, we present a brief description of the protein and polysaccharides nature, and chemical structure and describe the synthesis methodologies and their physicochemical characteristics taking into account the biomedical applications.

Chemical Structures of Collagen, Gelatin and Hyaluronic Acid

Collagen is the most abundant protein constituting more than one-third of weight of total body protein present in mammals. This protein has a complex hierarchical

CHAPTER 8

Approaches for Improving the Mechanical Properties of Collagen Biomaterials

María Victoria Tuttolomondo[1,2], Maria Lucia Foglia[1,2], Juan Manuel Galdopórpora[2] and **Martin Federico Desimone[1,2,*]**

[1] *IQUIMEFA-CONICET, Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Química Analítica Instrumental. (1113) Junin 956 Piso 3. Ciudad de Buenos Aires, Argentina*

[2] *Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Química Analítica Instrumental. (1113) Junin 956 Piso 3. Ciudad de Buenos Aires. Argentina*

Abstract: Collagen is a natural constituent of the extracellular matrix of tissues. It is well known that, when prepared without additives, diluted collagen hydrogels present poor mechanical properties limiting their use in tissue engineering. Hence, collagen's structural properties need to be enhanced. For this purpose, this chapter presents an overview of the recent advances and alternatives developed to improve the mechanical properties of collagen biomaterials.

Keywords: Biomaterial, Collagen, Crosslink, Fibrils, Hydrogel, Mechanical properties, Thermal stability, Tissue engineering, Wound healing.

INTRODUCTION

Within the biomaterials field, efforts are made in order to mimic the extracellular environment, taking into account its natural biocompatibility and mechanical properties. The collaboration between different disciplines is therefore sought to reproduce the complexity of the affected tissue. Among the different types of tissues found within the body, the connective tissue provides support and connects different tissues or organs. For its supportive function, the mechanical behavior is primarily determined by the composition and organization of the collagen present in it, a reason for which the characterization of fibril morphology and organization is so important in the design of new materials [1]. Herein, we present an overview of those recent approaches made to improve the mechanical properties of collagen involving the use of higher collagen concentrations as well as modifications in

* **Corresponding autor Martín F. Desimone**: IQUIMEFA-CONICET. Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Química Analítica Instrumental. (1113) Junin 956 Piso 3. Ciudad de Buenos Aires, Argentina; Tel/Fax: +54-11-49648254; E-mail: desimone@ffyb.uba.ar

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pH, temperature or ionic strength along with cross linking methods, all of which result in a significant enhancement of the thermal stability and rheological properties of the collagen network.

Biological Importance of Collagen and its Structure

Within the extracellular matrix, collagen molecules assemble into 25 to 500 nm fibrils, which further aggregate giving rise to fibers, the latter with a width up to hundreds of micrometers. Fibril width within a given tissue is associated with the capacity of it to withstand plastic deformation and high mechanical forces. While small fibrils allow the tissue to support plastic deformation, thicker ones are related to its ability to tolerate high mechanical loads. Additionally, glycosaminoglycans, hyaluronan and elastin are present in connective tissues in a smaller proportion when compared to collagen, though they have been found to contribute to the tissue´s overall mechanical stability. Oxlund *et al.*, investigated the roles of hyaluronic acid, collagen and elastin in the mechanical properties of connective tissues. By means of enzymatic degradation, they were able to measure the response of the digested specimens after a load was applied. As a result, stress-strain curves showed that collagen is the component which is mainly responsible for the tensile strength of skin and aorta [2]. In a later work, the sole contribution of elastin was assessed which suggested that elastin fibers might be responsible for the recoiling mechanism after a stress or deformation is applied [3].

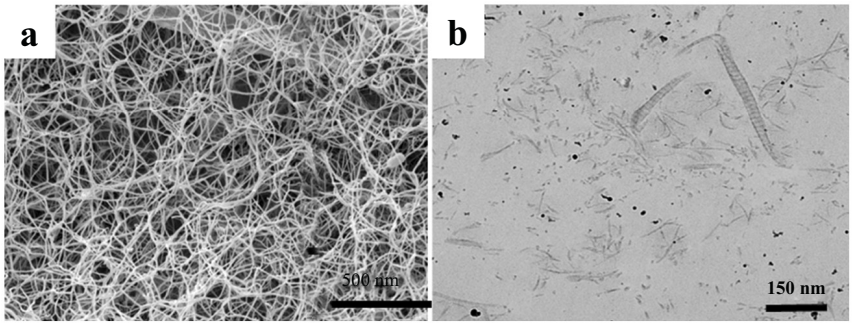

Fig. (1). a) representative SEM image of a collagen hydrogel network. **B**) TEM image where the typical cross striations are seen in the collagen fibre.

Type I collagen is present in connective tissues in concentrations ranging from 30 to 40 $mg.mL^{-1}$. In the presence of acid, the net charge of the polypeptide chain is altered and, as a result, hydrogen bonds that hold tropocollagen molecules together are disrupted leading to their release from the fibrils. After its solubilisation in diluted acid medium, collagen suspensions are obtained in concentrations which lay far behind from the ones normally found in living

tissues. Such collagen suspensions give rise, upon neutralization, to matrices or gels composed of around 60 to 100 nm fibrils with the characteristic axial D periodic spacing. The gel consists of a fibrous network (with fibers ca. 60 nm in diameter) exhibiting a high porosity level, with an average pore size larger than 200 nm, characteristic of these hydrogel structures. Moreover, the characteristic transversal cross-striations with a periodicity of 67 nm were clearly evidenced on TEM images (Fig. **1**). However, one of the main limitations of using these types of collagen gels as biomedical materials is related to their poor mechanical properties [4].

Properties of *in vitro* Reconstituted Collagen Matrices

in vitro, the mechanical properties of reconstituted collagen matrices have been extensively studied by evaluating the individual contribution of changes in the pH, temperature, protein concentration and ionic strength of different preparations [5 - 7]. In relation to this, Achilli *et al.*, prepared collagen gels under different conditions (pH, temperature and ionic strength) and observed that at pH 10, fibril packing was increased along with the mechanical resistance of the obtained gel, even when the fibril diameter decreased at a higher pH. Higher pH involves higher number of electrostatic bonds between fibrils, hence resulting in a gel with better mechanical properties [8]. On the other hand, differences in collagen concentration led to variations in fibril density but not in fibril diameter, which appeared constant around *ca.* 60 nm [7]. Even though fibril diameter remains unchanged, a denser collagen network is achieved as its concentration rises, leading to gels with improved mechanical properties.

Through the commonly used extraction methods, collagen concentrations around 2 $mg.mL^{-1}$ are obtained, which after proper solvent evaporation give rise to collagen solutions up to 40 $mg.mL^{-1}$. Recently, Hélary *et al.*, managed to control the self-assembly conditions and in this way, increased the collagen concentration up to 5 mg mL^{-1}. The higher collagen concentration resulted in lower cell-mediated contraction, increased cell proliferation, and superior *in vivo* integration [9, 10]. The so obtained matrices proved useful for the cell culture of human dermal fibroblasts as their secreted metalloproteinases were able to remodel collagen, stimulating cell migration within the scaffolds [11]. Manipulation of dense acid collagen solutions remains a challenge but neutralization is possible after their exposure to ammonia vapors [12]. Dense hydrogels with concentrations higher than 100 mg mL^{-1} were also recently obtained by injection, dialysis and combination of the two methods [13, 14].

Similarly, changes in temperature lead to changes in the mechanical strength. As a matter of fact, fibrils formed at low temperatures (*i.e.*, 20°C) have larger

CHAPTER 9

Protein Plastic Foams

Chanelle Gavin, Mark C. Lay*, Casparus J.R. Verbeek* and **Anuradha Walallavita**

School of Engineering, Faculty of Science and Engineering, University of Waikato, Hamilton, New Zealand

Abstract: The development of protein-based biopolymers has been driven by the increasing global demand for polymer products, the need for sustainable practice within this industry and the availability of low cost by-products, such as high protein content meals. The continuing development of new and existing protein-based biopolymers will enable these materials to help supplement the increasing global demand for polymer products and to develop new markets with their niche applications. To date various compositions of protein-based biopolymers have been successfully used to produce injection moulded articles, films and foams. Biopolymers typically display poor foaming behavior and commonly produce foams with irregular morphology and high densities. Protein-based biopolymers are no exception, therefore it is important to fully understand how the foaming mechanisms of bubble nucleation, growth and stabilization are affected by the inherently different properties of these materials.

This chapter aims to review the production of stable protein-based foams for use in applications such as cushioning, insulation and packaging through a variety of methods. The review specifically focuses on the production of protein-based foams through thermosetting, the emerging role of proteins as a renewable substitute in polyurethane production and the application of thermoplastic foam technologies to protein-based thermoplastics, with an emphasis on batch and extrusion foaming methods. The similarities and differences between the production of traditional foams and those produced from proteins are highlighted here. Discussion of foam morphologies, properties and processing conditions is also included. Overall, this chapter intends to provide the reader with a greater understanding of the existing research and the current challenges associated with the production of protein-based thermoplastic and thermoset foams.

Keywords: Batch foaming, Extrusion foaming, Polyurethane foams, Protein biopolymers, Protein foams, Thermoplastic proteins, Thermoset foams.

* **Corresponding authors Mark C. Lay and Casparus J.R. Verbeek:** School of Engineering, Faculty of Science and Engineering, University of Waikato, Hamilton, New Zealand; Tel: +64 7 856 2889; E-mails: mark.lay@waikato.ac.nz, johan.verbeek@waikato.ac.nz

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INTRODUCTION

This review examines the application of protein-based biopolymers to the polymer foam industry and discusses potential manufacturing methods for stable protein-based foams intended for use as packaging, insulation or cushioning. Until now, reviews regarding protein foaming have typically been limited to those relevant to the food industry with very little emphasis on their application to or use in thermoset or thermoplastic foams [1]. However with increasing interest in thermoplastic proteins for producing injection molded articles and films it is only logical that the industry also begins to assess the foaming ability of these materials on a wider scale.

The development of protein-based foams with similar or enhanced properties in comparison to their traditional polymer counterparts creates an entirely new market at the intersection of the polymer foam industry and the growing biopolymers sector. Recent reports regarding the polymer processing sector show that the global demand for polymer foam products is increasing, such that by 2019 it is predicted that the sector will generate 25 million tons of material. This represents an increase in production of nearly 32% on the 2013 figures, when the industry totaled 19 million tons (equivalent to $USD 87 billion) [2]. The majority of materials consumed by this sector are non-renewable and/or non-biodegradable including polyurethane, polystyrene and polyolefin materials such as polypropylene and polyethylene [2].

Traditional polymeric materials are being supplemented, and to some extent replaced, by biopolymers to address issues regarding the sustainability of this industry. The leading biopolymers are currently starch and PLA, however the use of protein-based biopolymers is also increasing, especially within the US [3]. Combined, this sector is expected to generate $USD 3.67 billion in revenue in 2018 [4, 5]. Unfortunately, the economics relating to the proportion of these materials which are foamed are as yet unpublished, hence the exact size and value of this market is unknown. Furthermore, a number of barriers exist for these and other biopolymer foams including consumer acceptance, poor thermal stability and the associated difficulty and cost of manufacture [6].

In general, the production of foamed polymeric products involves the introduction of a gaseous phase to the polymer system through chemical blowing agents (CBA) or physical blowing agents (PBA), the expansion or evolution of this gas due to changes in physical parameters such as pressure or temperature and the subsequent solidification of the resulting structure (usually by temperature changes or crosslinking) before the cells rupture or collapse due to condensation of the gaseous phase [7, 8]. Following stabilization the blowing agent is

eventually replaced by air as the gaseous species exchange across the cellular matrix (Fig. **1**). This phenomenon has been extensively studied and reviewed with respect to polyurethane, polystyrene, polypropylene and polyethylene foams where the behavior of these materials can be predicted relatively well in relation to physical foaming behavior; however the foaming mechanisms of bubble nucleation, growth and stabilization are yet to be fully understood.

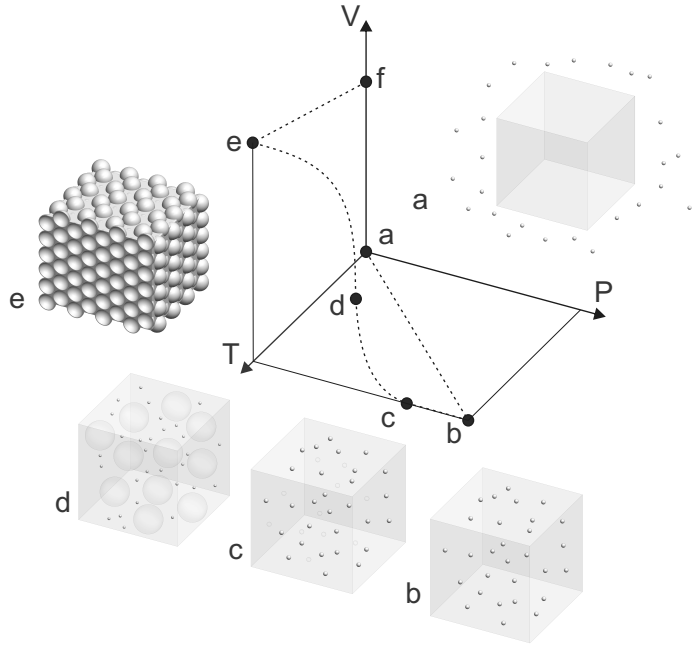

Fig. (1). Polymeric foaming process represented using a pressure, volume, and temperature diagram [7], with each stage shown by a letter and picture. The polymer and gas (**A**) is heated and pressurised to saturate the polymer (**B**), which is then depressurised so the gas changes phase and nucleates in the polymer (**C**), and as pressure drops, the remaining gas diffuses into the bubbles and the bubbles grow (**D**), until the final expanded foam is produced (**E**), and cooled (**F**).

Furthermore, the behavior of protein based biopolymers is still being characterized in relation to their structure, reactivity and processing ability. These are all related to their primary structure (amino acid sequence) and secondary structure (α-helices and β-sheets). Research is limited regarding the behavior of proteins within foaming systems. The properties of these materials, including poor rheological properties, low solubility of blowing agents and diffusion behaviour, severely hinder the foaming process. Consequently foamed biopolymers typically demonstrate non-uniform morphology and high density [6].

To date, efforts have been made to foam proteins through thermosetting methods

CHAPTER 10

Biopolymer Electrospinning

Athira K. Sunil*

Indian Institute of Science, Bangalore – 560012, India

Abstract: Electrospinning is a useful tool for producing fine fibers of about 10 nanometers to 10 microns in diameter from both natural and synthetic polymers. Historical developments, theory, and the types equipment used to produce aligned and non-aligned fibres in electrospinning are discussed. Collector composition or substrates, dimension of the collector, electrospinning materials and configurations of nozzle in electrospinning are discussed with their respective importance. The electrospinning process is influenced by the following solution parameters like viscosity, polymer molecular weight, polymer concentration, surface tension, polymer solution conductivity or surface charge density, rate of evaporation of solvents, dielectric constant and vapour pressure. Voltage, needle tip to collector distance, feed-rate, collector material, electrospinning setup, diameter of needle orifice, environmental (ambient) parameters like temperature, humidity, type of atmosphere and pressure also determine electrospun fiber size and morphology.

Keywords: Co-electrospinning, Collector, Electrospinning, Parameters, Spinneret, Voltage.

INTRODUCTION

Electrospinning is a versatile and cost-effective technique for producing multi-functional nanofibers from various polymers, polymer blends, composites, sol-gels, ceramics, *etc.* [1]. The fibres have diameters ranging from a few microns to tens of nanometres [2 - 5].

In a typical electrospinning set-up (Fig. **1**), a solution or melt is first fed through a spinneret with an inner diameter in the order of 100 μm. When a sufficiently high electric field is applied to the spinneret, which simultaneously serves as an electrode, the repulsive force caused by the concentration of similar charges in the solution can overcome the opposing surface tension of the droplet at the tip of the spinneret, and a Taylor cone is formed, from which a polymeric jet initiates.

* **Corresponding author Athira K. Sunil**: Indian Institute of Science, Bangalore – 560012, India; Email: athiraiisc@gmail.com

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Although the jet is stable near to the tip of the spinneret, it soon enters a bending instability stage with the formation of a whipping cone under the influence of the coulombic forces. This electrospinning jet stretches and coils, with an increasing diameter, until the solvent has evaporated, yielding fine fibres with diameters as low as a few tens of nanometres. The fibres are collected as a non-woven mesh of continuous fibres on an earthed collector, usually a metal plate or drum. The distance between nozzle and collector is set large enough to enable most of the solvent to evaporate and form dry fibres on the collection plate. The polymer chain entanglements within the solution prevents the electrospinning jet from breaking up and, depending on the stability of the electrospinning process, fibres with lengths of up to many hundreds of metres can be obtained.

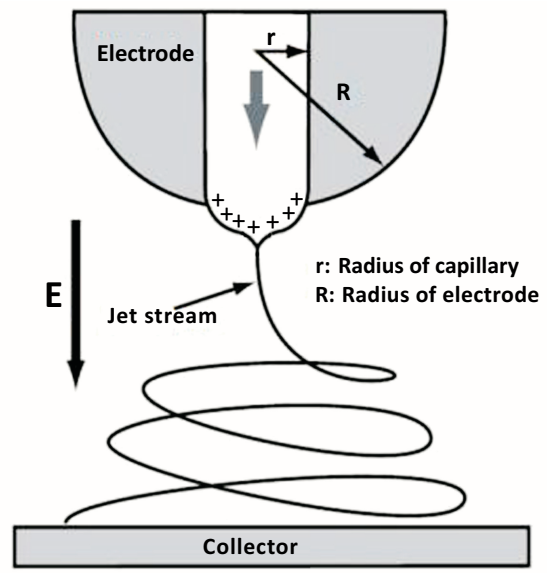

Fig. (1). Schematic diagram of an electrospinning apparatus and Taylor cones.

The versatility of the electrospinning process to produce fibres from a large range of polymeric materials or material combinations, including non-polymeric ones, either through mixing, co-axial or multiphase electrospinning of solutions, or through addition of dopants, provides the scope for this method to be used to fabricate fibres for many different applications. Post-processing of electrospun fibres, *e.g.* heat or solvent treatments for creation of pores in fibres, further increases the field of applications, with more novel functions being realized through the use of different materials.

History of Electrospinning

The discovery of electrical effects on liquids was carried out in the late 1500's when William Gilbert [6] set out to investigate the phenomena of electricity and magnetism. Gilbert observed that a spherical droplet of water was drawn into a conical shape when a charged piece of Amber was placed near it, which caused small droplets of water to be emanated; a phenomenon now called electrospraying.

It was not until 1898 that electrodynamics theory was used by Larmour [7] to explain the excitation of dielectric liquid under the influence of an electric charge. Four years later in 1902, Cooley [8] and Morton [9] patented the first devices to spray liquids by applying an electrical charge. The electrospinning in Coley's patent included a rotating collector for fibres produced from the jet directed by an auxiliary electrode [5].

Electrospinning was further investigated by Antonin Formhals who attained the first patent [10] on electrospinning in 1934 for the fabrication of textile yarns from cellulose acetate using acetone and monomethyl ether of ethylene glycol as solvents [11]. Formhals produced several patents for fibre production and collection, including designs that do not require a spinneret or rotating fibre collection devices [10, 12, 13]. Many of the recent electrospinning setups can in fact be traced back to these inventions and it is clear from these developments that the early researchers had already gained an in-depth knowledge of the electrospinning process [5].

However, it was not until 1964 that Sir Geoffrey Ingram Taylor [14], continuing work started by John Zeleny in 1914 [15], investigated the behaviour of fluid droplets under the influence of an electric field and initiated mathematical studies on the jet forming process [16 - 18]. Taylor described that the onset of the jet in the electrospinning process was initiated by the formation of a conical shape, now known as the Taylor cone. He theoretically derived that a perfect cone formed under the influence of an electric field required a semi-vertical angle of 49.3° and demonstrated that the angle of the cone on a droplet approached this theoretical value just before jet initiation. Two years later in 1966, Simons [19] patented the apparatus for the production of lightweight, ultra-thin, non-woven fabrics using electrospinning. In 1971, Baumgarten [20] reported electrospinning of acrylic fibres from a new set up that produced fibres with diameters in the range of 0.05–1.1 μm. Baumgarten showed how the diameters of the fibres changed with changing electric field. Despite these early discoveries, electrospinning did not gain a lot of interest and was not utilized commercially until the 90's. It is only since the emergence of nanotechnology in the 1990's that electrospinning gained

CHAPTER 11

Biopackaging: Tara Gum Films

Martin A. Masuelli* and **María Guadalupe García***

Instituto de Física Aplicada - CONICET, Universidad Nacional de San Luis, Chacabuco 917 (ZP 5700), San Luis, Argentina.

Abstract: In this work, we prepared and characterized water insoluble Tara gum films. Tara gum (TG) is a polysaccharide extracted from the endosperm of *Caesalpinia spinosa* seeds with a performance of 12% wt. TG films were evaluated as barrier material for agricultural and food industries. The films were modified in order to improve water resistance by using glutaraldehyde (Glu) as crosslinker. The crosslinking process consisted of placing the TG films in a bath containing Glu in an acidic medium for a period of 12 and 24 hours (TG-12 and TG-24) at 25ºC. Film properties were structurally studied through XRD, TGA and SEM images and operationally evaluated by measuring water vapor permeability (WVP), gas permeation and mechanical properties. WVP values decreased with increasing crosslinking time. This is due to crosslinking which hinders the diffusion of vapor molecules through the polymer matrix. Gas permeability tests showed permeabilities in the order CO_2>> N_2> O_2. Mechanical tests indicated an increase in the elastic modulus of the film with increasing crosslinking time; this effect is due to the loss of flexibility of the crosslinked polymeric matrix.

Keywords: Films, Gas permeation, Mechanical properties, Tara gum, Water vapor permeability.

1. INTRODUCTION

Plastic food packaging represents a threat to the environment all around the world due to the fact that they are derived from no renewable source and they are not biodegradable. Global production of packaging materials is estimated in more than 180 million tons per year, with growth and demand increasing annually. Within the plastic packaging market, food packaging is the largest growing sector suggesting the need for creating sustainable packaging alternatives, specifically the design and the development of biodegradable films [1]. The packaging serves several important functions: contains the food, protects from chemical, physical

* **Corresponding author Martin Masuelli and Mariaía Guadalupe García**: Instituto de Física Aplicada - CONICET, Universidad Nacional de San Luis, Chacabuco 917 (ZP 5700), San Luis, Argentina; Email: masuelli@unsl.edu.ar, maggarcia@unsl.edu.ar

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and microbiological deterioration, and provides practical means to inform consumers about the products [2 - 4]. In addition, the packaging preserves the shape and texture of the food, prevents flavor or aroma loss, prolongs storage time and regulates the water content or moisture of the food [5 - 7].

Recent advances in polymer technology promote the use of polymeric films for packaging foodstuffs. In this sense, there have been designed biodegradable materials from agro-industrial polymers obtained from renewable, abundant and low cost resources [8]. For example, cellulose is a biodegradable natural polymer of particular importance due to its application in plastic fibers [9]. From the total biopolymer production 41% is used for packaging industry and 47% of it is particularly applied in food packaging. Due to that, the use of biopolymers emerged as an excellent alternative to reduce existing environmental problems [10].

Certain food products such as coffee show metabolic processes even after packaging. These processes can lead to a faster degradation of food decreasing their useful life. Hence, characteristics of the packaging material play an important role in the food industry to extend the time of consumption. Among the various materials used, Tara gum films have been studied due to thier very good gas barrier and mechanical properties; however they are very permeable to water. In order to solve water permeability, crosslinking agents are often used [7].

Tara gum is a natural polymer obtained by grinding the endosperm of the seeds of *Caesalpinia spinosa* (Fam. Leguminosae); consists chiefly of polysaccharides of high molecular weight composed mainly of galactomannans. Tara gum has been used in food industry as gelling agent in juices, ice cream, sauces, pet food among others. Tara Gum seeds are contained in sheaths of 8 to 10 cm in length and four to seven seeds about 6 to 7 mm in diameter are present in each sheath. The shells of the seeds are about 25 to 27% of endosperm and 11% to 5% of moisture. Tara gum is a white, tasteless powder forming a viscous gel when mixed with water [11]. The viscosity of the solution depends on the chain length containing galactomannan. The hydrolysis with strong acids may reduce or abolish the viscosity of the gum.

Food packaging is concerned with the preservation and protection of all types of foods and their raw materials, particularly from oxidative and microbial spoilage and also to extend their shelf-life characteristics. Polymer cross-linking and graft copolymerization of natural polymers with synthetic monomers are other valuable alternatives in the design of biodegradable packaging. However, chemically modified biopolymers can loss biodegradability so degradation mechanisms of the films before their application need to be studied [12]. During the last decade, the

consumer pressure may trigger the use of biobased packaging materials as an alternative to materials produced from non-renewable resources. Biologically based packaging is defined as packaging containing raw materials originating from agricultural sources, *i.e.* produced from renewable biological raw materials such as starch and bioderived monomers. These materials are not necessarily biodegradable. Biobased packaging materials include both edible films and edible coatings along with primary and secondary packaging materials [13].

Galactomannans are preferred hydrocolloids since they are comparatively cheap, non-toxic, eco-friendly and non-polluting during production and application. Galactomannans from seeds of three species of Gleditsia, namely *G. sinensis, G. microphylla and G. melanacantha,* were characterized in terms of structural and thermal properties. Gleditsia polysaccharides consist of D-mannopyranose and D-galactopyranose residues. The mannose/galactose (M/G) ratio of Gleditsia galactomannans was 3.25, 3.31 and 2.30 for *G. sinensis, G. microphylla* and *G. melanacantha*, respectively. These values indicate that Gleditsia gums offer an excellent alternative to locust bean gum [14, 15].

Innovations constantly appear in food packaging, always aiming at creating a more efficient quality preservation system while improving food attractiveness and marketability. The utilization of renewable sources for packaging materials, such as hydrocolloids from biological origin, is one the main trends of the industry. Edible films/coatings have been considered as one of the potential technologies that can be used to increase the storability of foods and to improve the existent packaging technology, helping to ensure the microbial safety and the preservation of food from the influence of external factors. In view of these recent developments, the main objective of this review is to provide information concerning the utilization of galactomannans from Tara gum in the production of films. The most important features of these polysaccharides are discussed, namely: their structure and applications; physical, chemical, thermal and mechanical properties of galactomannan-based films/coatings; transport properties (in particular those related to moisture, oxygen, carbon dioxide exchange through the films/coatings); incorporation of active compounds (*e.g.* natural antimicrobials and/or antioxidants) and applications in food products. It is viewed that in a near future tailored edible packaging based on polysaccharides can be applied to selected foods, partially replacing non-biodegradable/non-edible plastics [16, 17].

Chemical structure of locust bean gum is shown in Fig. (**1A**). It is a galactomannan similar to guar gum consisting of a (1, 4)-linked β-D -mannopyranose backbone with branch points from their 6-positions linked to α-D -galactose (*i.e.* 1,6-linked α-D-galactopyranose). There are about 3.5 (2.8-4.9) mannose residues

CONCLUSIONS

At the end of this ebook, let us recall the tremendous efforts researchers have put on the development of techniques and methodologies for the molecular characterizations and the determination of the physicochemical properties of biopolymers from different sources in order to diversify as such as possible their end-uses for industrial applications. For that, researchers and engineers have adopted a strategy based on the understanding of the relationship between their structure and their macroscopic properties. Because biopolymers are able to generate macromolecular assemblies such as three-dimensional networks, two-dimensional films or one-dimensional monomolecular layers, in hydrated or solid forms, the structure of biopolymers alone or involved in such macromolecular assemblies have to be characterized at different scales, from the nanoscale to the macroscale including mesoscale, in order to deeply screen the structure-function-property relationships. We hope this first objective is reached in the present ebook.

Authors in this ebook have shown, through the diversity of the countries they belong, that biopolymers, from animal, plant, microbial and seaweed sources, can be judiciously extracted and purified according to as much as possible eco-friendly processes, in order to get reliable macroscopic end-use properties. Polysaccharides from plants, with cellulose being the most abundant on Earth, are the most used biopolymers for applications as their useful properties can be largely exploited for end-use applications. Natural gums from plants such as mesquite, tara and arabic gums are also very good candidates to promote economical development in countries where they originate from as they often are good stabilizers and adhesives for food and non-food products. Plant proteins are shown in this ebook as very promising alternatives to petroleum carbon sources for edible coatings and plastic foams.

The ebook displays also a thoroughly state-of-art in the physicochemical methods of characterization of biopolymers from the molecular level, through the exploration of size, polydispersity, chemical nature of repeating units, type of branching, using separation methods coupled to optical detectors but also mass spectrometry and NMR spectroscopy, to the macroscopic scale, through the exploration of the mechanical properties. A special attention is also given to the methods at the mesoscopic scale with the use of spectroscopic and microscopic methods in order to probe the structure covering the nanometric and micrometric scale. Small angle scattering and positron annihilation lifetime spectroscopy are methods highlighted in the ebook to probe nanostructures of single or complex biopolymeric materials.

The continuing development of new and existing biopolymers for end-uses applications are highly documented and we hope readers will find new opportunities for their teaching and research-development activities with this exhaustive list of biopolymers and related applications presented in the ebook. For instance, the design and engineering of innovative bionanocomposites and bioplastics deserve a special attention because it forms a fascinating interdisciplinary area that brings together biochemistry, materials science and nanotechnology. The understanding of the chemistry and structure at the nanometric scale imply an important knowledge for future developments of novel materials based on biopolymers and nanostructured materials. The extraordinary versatility of these new

materials comes from the large choice of biopolymers available. These new materials have been elaborated thanks to the developement of new powerful techniques described in the ebook such as electrospinning.

Revolution in biopolymers applications comes from the use of proteins and polysaccharides in nanostructured food and non-food materials with the elaboration of biocompatible and degradable micro and nanoparticles (with a solid or a liquid core) for active delivery applications and bioplastics for packaging applications. Therefore, the toolbox that micro- and nanotechnologies offer provides new opportunities for product and process innovations in the food and non-food industries. The control of the process and functionality at the nanoscale will lead to more sustainable food and non-food production. This approach will allow in food industry for instance the development of nutrient delivery systems with healthy and/or less caloric value nutrients, sensors, and diagnostic devices that can monitor and ensure the safety of food products throughout the food chain. At least, various enhanced packaging concepts will extend the shelf life of fresh products or indicate quality deterioration of the packaged product. Nevertheless, it is imperative to develop a good communication of the applications of biopolymers in the "nano world" that allows the consumers to make an informed decision whether or not they would like to have the benefits of certain applications of biopolymers in nanotechnologies, or whether they do not accept certain risks.

All these driving forces act as stimuli to develop new materials based on biopolymers, and there are many opportunity areas such as industrial, medical, food, consumer products and pharmaceutical applications for which biopolymers act as stabilizers, thickeners, binders, cross-linkers, dispersants, lubricants, adhesives, drug-delivery agents. We sincerely hope the readers will find stimuli-responsive answers to the huge diversity of bioplymers and their related applications in this ebook.

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Martin Masuelli

Martin Masuelli is a scientist at the Instituto de Física Aplicada – CONICET – UNSL, San Luis, Argentina. He holds a master's degree and a PhD thesis in Membrane Technology from the National University of San Luis (UNSL). He is a Coordinator of Physics Chemistry Area and a Director of Physics Chemistry Servise Laboratory, UNSL. He is an expert in polysaccharides and physics chemistry of macromolecules and has authored or co-authored more than 20 peer reviewed international publications, 5 book chapters, 65 communications in national and international congresses, and is an editor of one book ("Fiber Reinforced Polymers - The Technology Applied for Concrete Repair", INTECH, Croatia, 2013). He is a member of the Sociedad Argentina de Ciencia y Tecnología Ambiental and Asociación Argentina de Fisicoquímica y Química Inorgánica. He is also the Editor-in-Chief and in July 2013, became the founder of Journal of Polymer and Biopolymers Physics Chemistry, Science and Education Publishing. He is on board of numerous Journals.

Denis Renard

Denis Renard is a scientist at the Agronomic Research National Institute (INRA, France) and associated with the Nanostructured Assemblies. He holds a master's degree and a PhD thesis in physical chemistry of foods from the University of Nantes. He made a postdoc at CEA/LLB (Saclay, France) to study the behavior of biopolymers mixtures under shear by small angle neutron scattering. He is the author or co-author of more than 50 peer-reviewed international publications, 10 book chapters, 130 communications in national and international congresses, and editor of one book (Plant Biopolymer Science, RSC Publishing, 2002). He is a member of the Neutron French Society (SFN), the Bioencapsulation Research Group (BRG) and the French Synchrotron Radiation Users (AFURS). He now manages the microfluidic group in Nantes where he develops researches on new innovative biopolymers based microparticles and related drug active or stem cell encapsulation applications by droplets milli- and microfluidics.