PREFACE

Chitosan is a partially deacetylated derivative of chitin, a natural polysaccharide extracted from crustaceans, insects and certain fungi. Owing to its unique properties such as biodegradability, biocompatability, biological activity, and capacity of forming polyelectrolyte complex with anionic polyelectrolytes, chitosan has been widely applied in the food and cosmetics industry as well as the biomedical field in relation to tissue engineering, and the pharmaceutical industry relating to drug delivery. This book gathers current research from around the globe in the study of chitosan, including its manufacture, properties and usage across a broad spectrum.

Chapter 1 - Chitosan was initially discovered in the mid-18th century, but remained little-known until preliminary clarification of its crystalline structure in 1934 and further pioneering studies by Muzzarelli and Hirano aroused further interest. Discovered in molds, and commercially produced from crustacean shells, chitosan is now used in diverse applications. Due to the seasonal lulls in fishery industries and the still-growing demand for high quality chitosan, sources like mushrooms and other fungi are being re-evaluated. However, the crab shells currently used to make chitosan are waste materials of the fishery industry. Hence, chitosan production from fungi can only be economically competitive if waste mycelia from the industrial use of fungi as bio-catalysts in "white biotechnology" , or waste carbon sources, e.g. from food processing industries, are used as substrates for cultivating high chitosan-yielding fungi. Many fungi are known to produce sufficiently high amounts of chitosan for commercial production, and many treatments can reportedly enhance chitosan yields without applying metabolic or genetic engineering techniques. However, although there many potential sources, applications, and manufacturers, of chitosan - and many chemical and physical techniques have been established for its characterization and quality control - it is still very difficult to obtain chitosan that is fully standardized with respect to molecular weight and degree of deacetylation, especially for pharmaceutical research.

Despite this problem, the chitosan research "community" is still growing, accompanied by exponential growth in the annual number of publications on chitosan. Further, chitosan was initially almost entirely used in macro-scale applications, but in recent decades many micro- and nano-scale applications of chitosan in the form of nano-particles and composite materials have emerged, and current foci are largely on such small-scale uses.

This chapter considers: the economic values of chitosan itself and the raw materials that are potentially available for chitosan production (apart from the fishery industry); sources of substrates and potential chitosan-producing fungi; cultivation techniques that can be used to

increase chitosan production; extraction methods; and laboratory protocols that can be used to determine the quality of the extracted chitosan. In addition, current and future applications are summarised and some results from the authors' studies are presented on chitosan adsorption of copper and 17ß estradiol, and the application of chitosan-containing substrates for biomimetic coatings in tissue engineering.

Chapter 2 - Chitosan is the deacetylated form of chitin. Generally, the substance becomes soluble in dilute acids when the degree of deacetylation is more than 50%. The solubility of chitosan in dilute acids is often needed to be modified when specific drug release properties have to be tailored into the dosage form. Chitosan carries free amine functionalities on the deacetylated units and hydroxyl groups on the acetylated as well as deacetylated units. Derivatization by introducing small functional groups such as, alkyl or carboxymethyl groups can increase the solubility of chitosan at neutral and alkaline pH without affecting its cationic character. In addition, chitosan can be grafted with other molecules through covalent binding. The amino groups can be used for acetylation, quaternization, reactions with aldehydes and ketones, chelation of metals etc. The hydroxyl groups can lend to o-acetylation, H-bonding with polar atoms etc. Primary derivatization followed by grafting improves the solubility, antibacterial, antioxidant, chelating, complexing, bacteriostatic and adsorbing properties while maintaining its mucoadhesivity, biodegradability and biocompatibility. Functionalities can also be used for interaction of chitosan with ions.

Chapter 3 - Chitin and Chitosan are natural polymers belonging to aminopolysaccharides having interesting structural features for chemical modifications to generate novel properties, functions and applications. Despite its huge availability, the utilization of chitin has been restricted by its intractability and insolubility. Chitosan because of its improved solubility and enhanced functions and properties find innumerable applications because of its biocompatibility, biodegradability and non-toxicity together with its antimicrobial activity and low immunogenicity. This review covers the production, properties and applications of chitosan and their derivatives.

Chapter 4 - The results of studies of interpolyelectrolyte complexes of chitosan and different polyanions are summarised and described systematically. Specific properties of chitosan as polyelectrolyte are described. The general concept of the formation of polyelectrolyte complexes is developed. Separate parts of the review are dedicated to investigation of polyelectrolyte complexes of chitosan with:

-biopolyelectrolytes, including polysaccarides (plant, animal, bacterial polysaccarides and lipopolysaccarides), proteins, nucleic acids and also modified natural polyanions (carboxymethylchitin, carboxymetylcellulose, etc.),

-synthetic polyanions.

The data on application of polyelectrolyte complexes of chitosan in medicine and biotechnology, particularly for creation of hemocompatible, thromboresistant materials, bioconstruction materials for replacement of coverlets, blood vessels, bone tissue, immobilization of biological active compounds, non-viral vectors of genetic information transfer and so on are generalized.

Chapter 5 - *N*-carboxyethylchitosan (CECh) is a chitosan derivative. It preserves the valuable properties of its precursor such as biocompatibility and biodegradability. Moreover, CECh possesses some advantages such as larger variety of functionalities and solubility in neutral and alkaline medium. The gained up to date knowledge on the synthesis of CECh, its solution properties and biological behavior in respect to cells and pathogenic microorganisms

are emphasized in the chapter. Due to its amino- and carboxyl groups CECh behaves as a polyampholyte/polyzwitterion in aqueous solutions. This chitosan derivative self-assembles into nanoparticles in a pH range close to its isoelectric point. The conditions (pH of the medium, ionic strength and polymer concentration) and CECh molecular weight for nanoparticles preparation are described in details. Electrospinning represents a unique tool to produce nanofibrous materials that resemble the extracellular matrix and thus are promising for wound healing and tissue engineering applications. The fabrication of CECh-containing nanofibrous materials by electrospinning is described in the chapter. Similarly to the case of chitosan, the electrospinning of CECh from its aqueous solutions is rendered feasible by the presence of a non-ionogenic polymer. In addition, CECh has been used for preparation of organic/inorganic hybrid nanofibers containing superparamagnetic iron oxide or silver nanoparticles by combination of the sol-gel technique and electrospinning. Some properties and possible applications of these hybrids are detailed. As a polyampholyte CECh forms polyelectrolyte complexes (PECs) with polyacids and polybases. The pH-dependent formation of PECs; the preparation of hydrogel materials; as well as nanoparticles from CECh-based complexes are discussed in the chapter. Finally, the design of non-woven textiles by combining PEC formation and electrospinning is reported.

Chapter 6 - Chitosan is a rather abundant material with exquisite properties, which may be processed into a variety of materials including hydrogels, fibres, membranes, etc. The production of chitosan-based nanogels, also known as macromolecular miceles, has been successfully achieved using different techniques, which will be reviewed. This chapter covers the properties and applications of chitosan nanogels in the biomedical field, namely as a drug delivery vehicle for biopharmaceuticals. The main achievements and recent developments will be addressed.

Chapter 7 - Many newly designed therapeutic biomacromolecules, including protein-based drugs, are characterised by reduced capacity to permeate biological membranes and/or low stability in physiological environments. Over recent years, a major challenge of the pharmaceutical industry has concerned the necessary development of suitable non-injectable drug carriers that permit overcoming these limitations, opening the possibilities for the administration of the referred molecules through routes which are alternative to the parenteral. In this regard, nanocarriers have emerged as one of the most exciting tools to circumvent these drawbacks, mainly owing to their increased surface-to-volume ratio, which results in improved interaction with epithelial surfaces and, in some cases, in the ability to cross epithelial barriers. Moreover, nanoparticles further enable the protection of the encapsulated molecules, which retain their biological activity, permitting their administration through routes that were previously unviable. The compelling need to design biocompatible, biodegradable and non-toxic vehicles has turned polysaccharides into a very attractive class of materials. In this group, chitosan has reached a position of evidence, due to its interesting physicochemical and biopharmaceutical properties. Chitosan nanoparticles have in fact proven to be very effective vehicles for systemic mucosal protein delivery, demonstrating high capacity for protein association, enabling their protection from harsh environments and, owing to chitosan mucoadhesive character, improving the proteins' residence time in contact with the absorptive epithelia. Overall, chitosan nanocarriers have been demonstrating the ability to increase the bioavailability of encapsulated drugs. In this chapter, we present chitosan-based nanoparticles that have been proposed for the administration of proteins through mucosal routes such as the oral, buccal, nasal and pulmonary. More specifically, the

various techniques and materials applied on the elaboration of the carriers, along with chitosan, are analysed and the efficacy of these carriers is discussed, analysing the various mechanisms proposed for their effectiveness.

Chapter 8 - Chitosan is a natural biopolymer derived from chitin found in the cell walls of fungi, molds, yeasts and exoskeletons of crustaceans, mollusks, crabs and shrimps etc. Chitosan is generally partially deacetylated polymer of N-acetylglucosamine which is second most abundant polymer on earth after cellulose. Chitosan is biocompatible, biodegradable, non-toxic and mucoadhesive polymer which has attracted the attention of the scientists and researchers in the pharmaceutical and biomedical fields. Chitosan has many properties which make it a suitable candidate to be utilized in various applications. Besides being biosafe it possesses antimicrobial activity where it is effective against the gram negative bacteria and also possesses antifungal activities. It has wound healing property. It also finds application in the cosmetics and biomedical arena. Major impetus is being laid in the field of pharmaceuticals where the polymer is being utilized for formulation development and novel drug delivery research. The polymer has been deployed in the formulation of the extended release tablets and various sustained release matrix tablets which have been utilized for the treatment of gastrointestinal related ailments such as amoebiasis. The same has been used to formulate the biofilms which have been developed for wound dressings and in manifestations such as mucositis. Chitosan has been engineered to transform into novel drug delivery systems such as nanoparticles, microspheres, gastrointestinal patches, buccal patches etc. through a number of reported methods. There are a variety of published reports where the chitosan microspheres have been utilized for colonic delivery and colon associated malfunctions. The same have also been used for other gastrointestinal related manifestations such as in *H. pylori* infection, duodenal ulcers etc. Moreover, these have been utilized for targeted drug delivery for the cure of a particular diseased organ. In continuation to this the nanoparticles have been utilized for the delivery of the antigen and protein i.e. in vaccine delivery wherein the immunoadjuvant property of the polymer has been harnessed to increase the potential of the vaccine since chitosan also improves the transfection efficiency and hence the absorption of drug or protein is enhanced. The gastrointestinal patches and other delivery systems have also been developed against a variety of malfunctions. This chapter is directed towards the elucidation of the use and development of the chitosan in the drug delivery field and includes a detailed discussion of the same alongwith a focus on the future prospects of the polymer in drug delivery.

Chapter 9 - In case of targeting the drug to the desired part of the skin, vehicles play an important role, beside the characteristics of the drug. Many natural and synthetic vehicles have been used for various topical dermal/transdermal preparations. However, chitosan has been standing out with its many advantages based mainly on its biological and physicochemical properties. Chitosan is a unique hydrophilic biopolymer obtained by partial deacetylation of chitin, which is one of the most abundant polysaccharide. It is a natural product widely found in crustacean shells, fungal cell walls, insect exosceletons, and mollusks. Chitosan is a linear glycosaminoglycan made up of N-acetyl-D-glucosamine units.

Characteristics of chitosan, such as the molecular weight, viscosity and the degree of deacetylation, greatly influence the properties of formulations. The by-products formed after the biodegradation of the polymer does not cause immune responses making it biocompatible. Due to the specific cationic glucosamine groups of chitosan, it can be interacted with anionic proteins in the skin providing the bioadhesive characteristics. These properties result in

improved efficacy, enhanced bioavailability and reduced toxicity -generally recognized as safe (GRAS). Furthermore, the antimicrobial/ antibacterial and skin hydrating effects of chitosan have been received considerable attention for dermal/transdermal applications. It plays an important role in the cell regulation, tissue regeneration and collagen production. Chitosan and some of its complexes were approved by FDA for use in wound dressing products.

Chitosan also provides the controlled release of numerous active agents used for the treatment of skin diseases such as corticosteroids, antifungal agents, nonsteroidal anti-inflammatory drugs, hormones, local anesthetics, antiviral and antiseptic agents, etc. Regarding to the good bioadhesive property of chitosan and its ability to sustain the release of the active compounds, it has found many practices in the formulation of gels, dermal/transdermal patches, sponges, micro- and nanoparticulate systems as drug carriers. Particularly, chitosan has been used in the preparation of mucoadhesive formulations, for improving the dissolution rate of the poorly soluble drugs, drug targeting and enhancement of peptide absorption.

This paper is focused on the use of chitosan for dermal/transdermal drug delivery systems following a general overview of chitosan. This natural polymer is a promising carrier or excipient as a delivery system and remarkable advances have been made about its potential applications in skin delivery.