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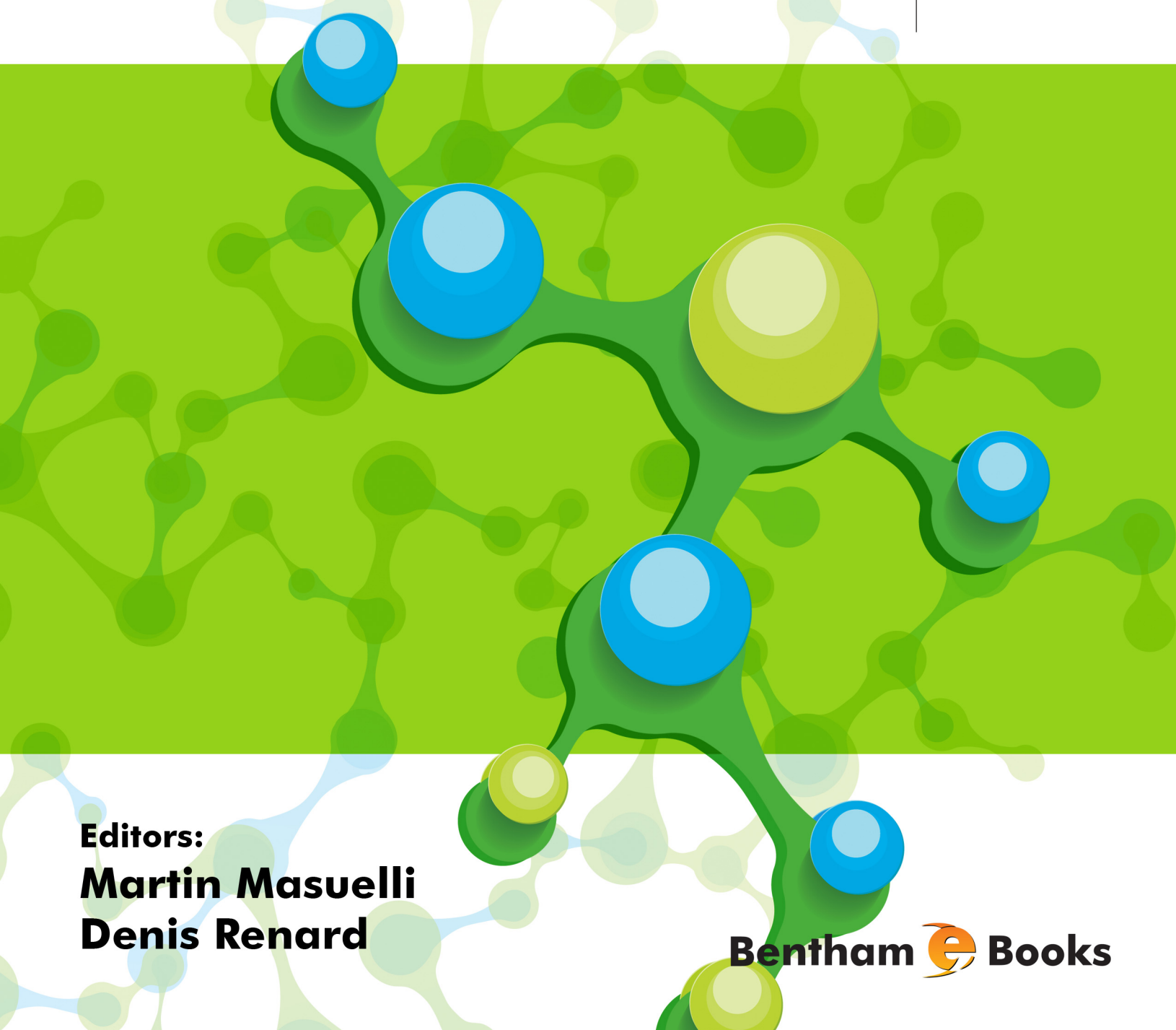
ADVANCES IN PHYSICOCHEMICAL PROPERTIES OF BIOPOLYMERS

Part 1



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Advances in Physicochemical Properties of Biopolymers

(Part 1)

Edited by:

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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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SECTION A. INTRODUCTION

CHAPTER 1

Molecular Weight and Molecular Weight Distribution for Biopolymers

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Abstract: In this chapter, molecular weight (M), and molecular weight distribution (MWD), of polymers with emphasis on M and MWD of biopolymers, *e.g.*, carbohydrate polymers, proteins, deoxyribonucleic acid, DNA, and ribonucleic acid, RNA, are reviewed. The M and MWD of biopolymers are compared with those of synthetic polymers. The following conclusions are drawn. (1) Unlike simple low molecular substances, most polymers do not have unique molecular weights. Practically, no polymer exists whose molecules are all strictly of the same size or have the same degree of polymerization. Thus, polymers are more or less heterogeneous with respect to their molecular weights. (2) The concept of average molecular weight is used for polymers. (3) Different numerical values for molecular weights of polymers have already been defined as average molecular weights (M_n, M_w, M_z, and M_v), depending on the methods by which they are measured. (4) The average values vary in the following order: $M_n < M_v < M_w < M_z < M_{z+1}$. The disparity between average molecular weights provides a measure of the degree of heterogeneity, *i.e.* dispersity, in the molecular weight distribution. (5) The constitution of a polymer as well as the MWD may be described either by a set of different average molecular weights, the ratios of two different types of average molecular weights, or by the distribution functions *via* graphical presentation and (6) Polysaccharides in a similar way to synthetic polymers are polydisperse polymers, whereas proteins, DNA, and RNA, are mostly monodisperse macromolecules.

Keywords: Biopolymers, Dispersity, DNA, Heterogeneity, Molecular weight, Molecular weight distribution, Polysaccharides, Proteins, RNA.

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INTRODUCTION

A macromolecule, known as a giant molecule or a polymer, is a chemical species, composed of a long chain with a regularly repeating unit, a high molecular weight and a high molecular size [1, 2]. The unit for molecular weight is usually the Dalton (Da); one Dalton is equal to one atomic mass unit. Symbols and parameters appearing in this chapter are given in Table **1**. Macromolecules are divided into natural and man- made polymers. The latter are known as synthetic polymers [3].

Table 1. Constants and symbols used in this chapter.

Symbol	Definition
M	Molecular weight
M_w	Weight-average molecular weight
M_n	Number-average molecular weight
M_z	Z-average (centrifuge average) molecular weight
M_{z+1}	(z+1)- average molecular weight
M_v	Viscosity-average molecular weight
[η]	Intrinsic viscosity in a solvent
K	Intercept for MHS equation
a	Exponent for MHS equation,
MHS	Mark-Houwink –Sakurada
q_{MHS}	Dispersity correction factor
$f_w(X)$	Cumulative weight fraction
X	Parameter characterizing the chain length
W(M)	Weight distribution function
N(M)	Number distribution function
a_1	Positive adjustable parameter
b_1	Positive adjustable parameter
μ	Mean value
σ_n	Standard deviation
K_{av}	Distribution coefficient
V_R	Solute elution volumen
V_0	Void volumen
V_C	Total bed volumen
b	Constant, it is an empirical polynomial function of MHS exponent *a*

(Table 1) contd.....

Symbol	Definition
c	Constant, it is an empirical polynomial function of MHS exponent *a*
N_{Av}	Avogadro ' s constant
S	Sedimentation constant
D	Diffusion Constant

This chapter focuses on natural polymers, also known as biopolymers.

Natural polymers, are produced by biosynthesis in nature, whereas synthetic polymers are made and their synthesis controlled by human beings. Biopolymers may be classified into proteins, nucleic acids, polysaccharides, and others. In this chapter, three main groups; polysaccharides, proteins and nucleic acids, which play important roles in all biological phenomena and processes are discussed [4 - 6]. A wide variety of natural polymers relevant to the field of biomaterials, is derived from plants and animals [5, 7]. Generally, biopolymers consist of carbohydrate polymers, proteins, deoxyribonucleic acids (DNA), and ribonucleic acids (RNA). They are fundamental to the biological substance of life [5]. Abbreviations and expressions are listed in Table **2**.

Table 2. Abbreviations used in this chapter.

Abbreviation	Expression, Term
CLND	Chemi-Luminescent Nnitrogen Detector
Da	Dalton
DI	Dispersity Index
DNA	Deoxyribonucleic Acid
DRI	Differential Refractive Index
GPC	Gel Permeation Chromatography
IUPAC	International Union of Pure and Applied Chemistry
RNA	Ribonucleic Acid
SEC	Size Exclusion Chromatography
MS	Mass Spectrometry
MWD	Molecular Weight Distribution

GENERAL CONSIDERATIONS

Carbohydrate macromolecules are known as polysaccharides. Monosaccharide units join together *via* glucosidic linkages and form a polysaccharide [7]. Proteins are linear polymers formed by linking the α-carboxyl group of one amino acid to

SECTION B. CHARACTERIZATION

CHAPTER 2

Intrinsic Viscosity Bovine Serum Albumin in Aqueous Solutions: Temperature Influence on Mark-Houwink Parameters

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Abstract: Bovine serum albumin (BSA) in aqueous solution is scarcely studied, and the Mark-Houwink parameters from the intrinsic viscosity measurements have not been reported at different temperatures. This work discusses these with a simple calculation of the Mark-Houwink parameters of BSA in aqueous solution when the concentration ranged from 0.2 to 1.0% wt., and the temperature ranged from 20 to 45°C. The relationship between the concentration and intrinsic viscosity was determined according to different methods. It is well known that when the temperature increases, the intrinsic viscosity decreases. This is reflected in the stiffer chain curve with d(ln[η])/d(1/T) of -398.97 for A zone from 20-30ºC (gel zone), -2759.1 for B zone from 35-40ºC (active zone) and 5604.5 for C zone from 41-45ºC (denatured protein zone), the point of intersection between the zones A and B is 34.6ºC. The linear relation between the logarithmic of viscosity and reverse temperature is ΔE_{avf} with a value of 680 Cal/mol. Furthermore, this work proposes the determination of M-H parameters of a protein-water system and their thermodynamic implications in conformational changes.

Keywords: BSA, Intrinsic viscosity, Mark-Houwink parameters.

INTRODUCTION

Bovine serum albumin (BSA) is a serum albumin protein derived from cows; the most abundant plasma protein is a globular protein. BSA is a major contributor to oncotic pressure (also known as colloid osmotic pressure) of plasma, acting as a carrier for various substances. Albumin is a soluble, monomeric protein which

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comprises about one-half of the blood serum proteins. Albumin functions primarily as a carrier protein for steroids, fatty acids, and thyroid hormones and plays an important role in stabilising the extracellular fluid volume.

Albumin is a globular non-glycosylated serum protein with a molecular weight of 66,500 g/mol. Albumin is synthesised in the liver as proalbumin, which has an N-terminal peptide that is removed before the nascent protein is released from the rough endoplasmic reticulum. The product, proalbumin, is in turn cleaved in the Golgi vesicles to produce the secreted albumin. Albumin (when ionised in water at pH 7.4, as found in the body) is negatively charged. The glomerular basement membrane is also negatively charged in the body; some studies suggest that this prevents the filtration of albumin in the urine. According to this theory, the charge plays a major role in the selective exclusion of albumin from the glomerular filtrate. A defect in this property results in nephrotic syndrome, leading to albumin loss in the urine. Nephrotic syndrome patients are sometimes given albumin to replace the lost albumin. Because smaller animals (for example rats) function at a lower blood pressure, they need less oncotic pressure to balance this, and thus need less albumin to maintain proper fluid distribution. The general structure of albumin is characterised by several long α (alpha) helices, which this allows it to maintain a relatively static shape, which is essential for regulating blood pressure. Serum albumin contains eleven distinct binding domains for hydrophobic compounds. One heme and six long-chain fatty acids can bind to serum albumin at the same time [1].

Table 1. Physical properties of BSA [8], from data sheet of SIGMA ALDRICH, Germany.

Number of amino acid residues	**Molecular weight (g/mol)**	**Stokes Radius (R_s)**	**Estimated α-helix, %**	**pH of 1% Solution**
583	66463 Da	3.48 nm	54	5.2-7
Isoelectric point in water at 25 °C	Dimensions	Intrinsic viscosity (cm^3/g)	Estimated β-form, %	Diffusion constant, $D_{20,W} \times 10^7$ (cm^2/s)
4.7	$140 \times 40 \times 40$ $Å^3$ (prolate ellipsoid where a = b < c)	4.13	18	5.9

In solution, BSA presents a versatile conformation modified by changes in pH, and ionic strength, which serves to characterise the structure, conditions and properties of BSA (see Table **1**). Conformational changes induced by pH are reversible [2]. Although there have been speculations on the possible function of each transition, its physiological meaning still remains unclear. Foster [2]

classified conformers as: “E” for expanded, “F” for fast migration, “N” for normal dominant form at neutral pH, “B” for basic form and “A” for aged at alkaline pH. Molecular weight, intrinsic viscosity, and Mark-Houwink parameters of BSA are shown in Table **2**.

Table 2. Intrinsic viscosity at different temperature.

Solvent/Solution	pH	T (ºC)	M_w (g/mol)	[η] (cm^3/g)	*a*	Reference
Water, 0.1M Sodium citrate	7.4	37		4.20	-	Hess & Cobure 1950 [9]
Water, 0.2 M NaCl	7	15 20 25 30	64540	4.12 4.17 4.18 4.06	-	Koenings & Perrings 1952 [10]
Water, 0.010 M KCl	-	25	65000	3.4-3.8	-	Tandford & Buzzel 1954 [11]
Water I=0.1	-	25	65000	3.8	-	Yang & Foster 1956 [12]
Water I=0.2, Na_3PO_4. Urea (8M), sodium borate (0.05 M). Sodium p-chloromercuribenzoate (0.005M)	7 8.3 9.9	30	66706	4.20 4.10 20.8-30.5	-	Charlwood 1955 [13]
Water, 0.15 NaCl	5	25	-	3.70	-	Tanford *et al.* 1955 [14]
Water I=0.1	2.03 3.06 3.73 2.83	60	-	5.1 8.6 10.7 4.8	-	Foster & Yang 1955 [15]
Water 0.5M KCl	4 5.13	25	67000	4.57 4.13	-	Loeb & Scheraga 1956 [16]
Water 0.15M NaCl	3 4.3 4.8 4.9 7.3 8.5 9.3 10.5	25	67000	6.50 3.74 3.76 3.71 3.68 4.17 4.00 4.00	-	Tandford & Bussel 1956 [17]
Formic acid, Formic acid-0.1M NaCl.	-	-	70000	31.8 21.6	-	Martin 1961 [18]
Water	3.6	25	66000	5.44	-	Bloomfield 1966 [19]

CHAPTER 3

Small Angle Scattering and *ab initio* Modeling

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Abstract: Small-angle scattering of X-rays (SAXS) or neutrons (SANS) is a well-established method to study the overall structure and structural transitions of biopolymers in solution. Determination of overall structural parameters such as the radius of gyration R_g, the molecular mass M_w or the hydrated volume V is now easily computed using appropriate free Softwares. In addition, increasingly sophisticated *ab initio* methods were developed over the last two decades for building structural models of biopolymers from x-ray and neutron solution scattering data. Even if most of the developments were focused on protein in solution, folded or intrinsically disordered, protein complexes or protein fibrils, *ab initio* methods were also used to calculate three-dimensional shape models of polysaccharides or nucleic acids. The models derived from experimental scattering pattern up to a resolution of 0.5 nm are classified as low resolution models compared to atomistic resolution structures using X-ray crystallography. The efficiency of the methods is illustrated in this chapter by focusing mainly on proteins or polysaccharides with unknown crystal structures and for which the *ab initio* reconstruction of three-dimensional models may help to define, in combination or not with other structural techniques, such as microscopy, overall dimensions and global shape. These methods improved the resolution and reliability of models derived from scattering data substantially and has made solution scattering, in combination with recent developments using size-exclusion chromatography, robotic sample changer or microfluidics, and a useful technique in high-throughput large-scale structural characterization of biopolymers.

Keywords: *Ab initio*, Complexes, Fibrils, Flexibility, Microfluidics, Modeling, Neutron, Pair distance distribution function, Polydispersity, Polysaccharide, Proteins, Radius of gyration, Sample changer, Size exclusion chromatography, Small-angle scattering, Three-dimensional structures, X-ray.

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INTRODUCTION

Small-angle scattering (SAS) of X-rays (SAXS) or neutrons (SANS) is a fundamental tool in the structural characterization of biopolymers with molar masses ranging from a few kDa to several MDa [1]. Great progress has been made over the years in applying this technique to extract structural information from non-crystalline samples in the fields of physics, materials science and biology [2]. Over the last decade, major advances in instrumentation and computational methods have led to new and exciting developments in the application of SAS to structural biology [3]. In particular, the synergistic improvement in hardware as well as software resulted in an automated data collection and in-depth analysis of the scattering data [4 - 6]. The absence of radiation damage and possibility of contrast variation by solvent exchange (H_2O/D_2O) or specific deuteration makes SANS an extremely useful complementary tool for SAXS [7]. SAS experiments delivers structural information in the length scale ranging from 1 nm to 1 μm depending on the instrument and source used (neutron or X-ray), and the largest length scale being explored by using ultra small-angle scattering (USAS). SAS experiments can be fast in particular on a third generation synchrotron and require moderate amount of purified material (order of hundreds of micrograms to a few milligrams, depending on the SAS instrument and the source used). For moderate polydisperse samples, the coupling of size-exclusion chromatography (SEC) to on-line SAXS may help in the structural determination of each component present in the sample. Structural changes due to environmental conditions (pH, ionic strength, temperature,…) can be easily monitored and directly correlated with functional results (*i.e.* kinetics, spectroscopy or interactions studies in solution) [8]. SAS is also extremely useful in the quantitative characterization of mixtures and flexible systems, including time-resolved experiments to monitor assembly or folding processes. Weak protein-ligand interactions and structural modeling of transient complexes are also widely studied using SAXS [9].

Solution structure will be investigated in this chapter to characterize overall parameters of shape and dimensions of isolated or interacted biopolymers alone or in interactions but readers should keep in mind that due to the high penetration of X-ray or neutrons, (nano)-structures on solid or gelled samples may also be explored using this technique. The focus will be on *ab initio* modeling methods to probe into the three-dimensional structure of non-crystalline biopolymers and the characterization of intrinsic flexibility encountered particularly in proteins. Special care will be given to the analyses of the polydispersed samples where developments have been brought these last years in the on-line combination of size exclusion chromatography to SAXS measurements. Kinetics using rapid mixing techniques will be presented with a focus on microfluidics as an emerging tool to probe structural dynamics of biological samples. Finally, some

developments dealing with the high throughput applications of SAS experiments, including combination of automated microfluidic platform, will be presented, generating a renewed interest in the wider applications of the technique in the structural biology community [3].

Basics of Small Angle Scattering

In a standard scattering experiment, the rotationally averaged scattering intensity of a biopolymer is obtained as a function of the scattering vector or momentum transfer, q:

$$q = \frac{4\pi sin\theta}{\lambda} \quad \textbf{(1)}$$

Where θ is half of the angle between the incident and scattered radiation and λ is the incident radiation wavelength. The SAS scattering intensity $I(q)$ can be expressed as a product of the form factor $P(q)$, which contains information on the shape and dimension of the scattering particles, and the structure factor $S(q)$ describing the interparticle interaction [10]:

$$I(q) = N\Delta\rho^2 P(q)S(q) \quad \textbf{(2)}$$

Where N is the number density of the particles, and $\Delta\rho$ = (ρ-ρ_0) is the so-called "contrast" (*i.e.* electron or neutron density, showing the difference between the scattering length density of the particle ρ and that of the solvent ρ_0). Using contrast variation by SANS, multiple data sets are recorded from the same object, which are different because of different contrasts of the components in the particle. The scattering length density of the particle ρ may be changed by selective deuteration or perdeuteration (*i.e.* deuteration in which all hydrogen atoms are replaced by deuterium) while ρ_0 is conveniently varied in a broad range by H_2O/D_2O exchange in solution [7]. Chemical method (for cellulose) [11] and perdeuterated algae culture (for phycocyanin) [12] were previously used for selective deuteration or perdeuteration to modulate $\Delta\rho$ until the developments of genetic engineering over the last fifteen years that now offers a large panel of cultured strains to produce partially or fully deuterated biopolymers such as polysaccharide [13], lipids [14], ribosomes [15], RNA [16], proteins [17] or complexes [7, 18]. In addition, modern neutron scattering facilities in the world (Institut Laue Langevin, Grenoble, France; Oak Ridge National Laboratory, Oak Ridge, USA; OPAL, Lucas Heights, Australia,…) offer the opportunity of deuterium labeling for structural biology studies. The other way to change $\Delta\rho$ and

CHAPTER 4

High-Performance Size-Exclusion Chromatography coupled with on-line Multi-angle Laser Light Scattering (HPSEC-MALLS)

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Abstract: High-Performance Size-Exclusion Chromatography (HPSEC) is widely used for the determination of the molar mass and size distribution of biopolymers in aqueous or organic solvents. Elements of the theory of fractionation by HPSEC, column calibration and online light scattering detection are given, showing that coupling HPSEC with multi-angle laser light scattering detection makes it easier to obtain molar mass distributions since light (MALLS) scattering gives the weight-average molar mass at each elution volume of the chromatogram. Some applications of HPSEC-MALLS for the macromolecular characterization of starches, glycogens, dextrans, celluloses, hemicelluloses, β-glucans, pectins, gums, alginates, carrageenans, chitosans, lignins, proteins and peptides are also presented.

Keywords: Cellulose, Chitosan, Gum, High-Performance Size-Exclusion Chromatography, Lignin, Multi-angle laser light scattering, Polysaccharide, Protein, Starch, Static and dynamic light scattering.

INTRODUCTION

Biopolymers found in nature are generally highly polydisperse. Their size and molar mass distributions are directly linked to their synthesis conditions and have an impact on their properties and their behavior in terms of degradation and other modifying processes. To understand their behavior, it is therefore important to determine these distributions. Size-exclusion chromatography (SEC, or gel permeation chromatography, GPC) is a very popular technique used for the determination of the molar mass distribution of polymers. It is a liquid chromatographic technique that allows the fractionation of molecules as a

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function of their hydrodynamic volume, *i.e.*, their size (which is proportional to their molar mass), by eluting across one or several columns filled with a porous stationary phase. The development of high-performance liquid chromatography leads to the appearance of high-performance SEC (HPSEC), which is highly precise (repeatable), robust and automatic. Since this is a size fractionation technique, the molar mass distribution is obtained by calibration of the system with standards. However, the relationship between size and molar mass is not that simple, depending on the macromolecules (in particular, in the case of branched ones), and is affected by operating conditions. That is why the development of HPSEC coupled with multi-angle laser light scattering (MALLS) detection is a real added value for the determination of molar mass distributions since light scattering gives the weight-average molar mass ($\overline{M}_w$) at each elution volume of the chromatogram. In this chapter, a brief overview of the theory of fractionation by SEC and online light scattering detection is first given, followed by some elements on column choice and applications of HPSEC-MALLS for the characterization of some biopolymers such as polysaccharides and proteins.

SEC THEORY

Fractionation Mechanism

During elution, molecules that are too large to enter the pores of the stationary phase, are eluted in the interparticular volume of the system (or total exclusion volume, V_0), whereas molecules that are sufficiently small to freely diffuse in and out of the pores are eluted in the interparticular volume of the system (or total column volume, V_t), equal to $V_0 + V_p$, where V_p is the pore volume. Intermediate-size molecules enter the pores and are separated by decreasing their size; the smaller they are, the more molecules enter the pores. Elution volume is expressed as follows [1]:

$$V_i = V_0 + K_{SEC}\, V_p \qquad \textbf{(1)}$$

$$K_{SEC} = \frac{c_p}{c_0} \qquad \textbf{(2)}$$

V_p is the accessible volume, K_{SEC} is the distribution coefficient, c_p is the mean concentration of the polymer in the pore volume, and c_0 is the interstitial volume. In the case of a pure SEC mechanism, *i.e.*, based on size only, $0 < K_{SEC} < 1$. A $K_{SEC}=0$ corresponds to an elution in the total exclusion volume, and when $K_{SEC}=1$, the solute is eluted in the total volume. If $K_{SEC} > 1$, the separation is controlled by enthalpic interactions that depend on the chemical composition of the polymer.

SEC Calibration

The aim of calibration is to obtain a relationship that links the elution volume to the molar mass of a sample. This technique is useful when only the concentration is determined online. The column calibration must be done in the same chromatographic conditions: eluent, temperature, elution flow, injected concentration and volume.

The classical calibration technique is based on the analysis of a series of monodisperse standards with known molar mass and the same chemical nature as the sample to be analyzed. The calibration curve shows the plot of the logarithm of the molar mass (*logM*) of the standards *versus* the elution volume (V_i), and must cover the entire separation range, from the excluded volume to the total volume [1, 2]. An alternative method is the use of polydisperse standards [1, 3, 4]. These two methods require standards of the same type as the sample because the size and the hydrodynamic volume depend on the length of the polymeric chains, their structure (linear or branched), their conformation (sphere or rod) and the solvent. That is why these methods are generally difficult to use, in particular, for branched samples since the standard structure must be exactly the same as the sample.

The universal calibration method was introduced by Benoit [5, 6]. It uses the hydrodynamic volume, V_η:

$$V_\eta \propto [\eta]\, M \tag{3}$$

where *[η]* is the intrinsic viscosity and *M* is the molar mass. The calibration curve, *log[η]M versus* V_i, is obtained with a series of monodisperse standards with known molar mass and intrinsic viscosity. The molar mass is then calculated with the Mark-Houwink-Sakurada (MHS) equation:

$$M = \left(\frac{V_\eta}{K_a} \right)^{1/a+1} \tag{4}$$

where *a* and K_a are constants, also referred as the MHS parameters. The advantage of this technique is the possibility to use standards with different chemical natures. However, it is valid only in the case of a pure exclusion effect, and the MHS parameters of the standards and the sample have to be known. More information can be found in the review of Kostanski *et al.* [7] where the different SEC calibration methodologies for simple and complex polymers were compared.

CHAPTER 5

Field-Flow Fractionation (FFF)

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Abstract: Field-Flow Fractionation techniques (FFF) are size-based separation methods first described in 1966 by Giddings. They belong to the family of liquid chromatographic techniques, but they are operated without any stationary phase.Yet, they have the unique ability to separate an extremely broad range of molecules, macromolecules and particles, and in particular very large particles, with a high resolution. FFF are versatile: by varying the experimental conditions, the range, speed, and power of the separation could be optimized. FFF techniques can succeed when Size-Exclusion Chromatography (SEC) methods fails, and they have a broad range of applications. In this chapter the theory of FFF is approached together with calibration and determination of some structural parameters such as size and molar mass, the instrumentation and detection of various classic FFF types is described and we show the added value of FFF techniques for the characterization of various biopolymers including polysaccharides, proteins and natural rubber.

Keywords: Aggregation, Field-Flow Fractionation, Multiangle Laser Light Scattering, Natural Rubber, Polysaccharides, Proteins, Starch, Static and Dynamic Light Scattering

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INTRODUCTION

Characterization of biopolymers, from polysaccharides to proteins, is one of the main challenges in biotechnology. Analytical methods should not only give accurate information on the molecule structure, but also have to preserve and investigate its native conformation and supra-molecular, non-covalent interactions. Size-Exclusion Chromatography (SEC) is a routine and a well-established analytical separation method but it has limitations and pitfalls for the analysis of large, polydisperse, complex and branched polymers or biological extracts. For example its large internal surface area sometimes produces interactions, shear degradation, poor analysis recoveries and anomalous elution of branched polymers and it requires preinjection filter (see Chapter 4). An Alternative method is Field-Flow Fractionation (FFF) which was introduced in 1966 [1] and operates in an open channel thus allowing a gentle separation mechanism, without extensional shear and possible separation in native sample environnement.The rate of scientific publications on effective application of FFF to biopolymers and their assemblies is constantly increasing. FFF coupled with multi-detection is indeed a powerful tool for obtaining information on macromolecular structure, such as size and molar mass distributions and conformation, which are all important features for complex biopolymers. This chapter presents first a brief overview of the theory of fractionation by FFF, followed by calibration and determination of some structural parameters, instrumentation and detection of various classic FFF types. Following this, the applications of Sedimentation FFF, Thermal FFF and Flow FFF for the characterization of biopolymers and bioassemblies based on polysaccharides, proteins and natural rubber are developed.

THEORY OF FIELD-FLOW FRACTIONATION (FFF)

The expression FFF encompasses a variety of separation methods which all share the same basic separation principle. They are indeed based on the coupling of a non-uniform flow of a carrier liquid with a force field applied orthogonally to the main flow direction in a conduit filled with the carrier fluid [1]. They differ by the nature of the applied field and are named according to the nature (Fig. **1**). In addition, they may also differ by the geometrical configuration of the separation conduit. Most frequently, the conduits have parallel plate geometry. In some cases (particularly in hollow fiber flow FFF, HF5), a tubular (cylindrical) geometry is used. The fluid flow, in isothermal systems, has a parabolic velocity profile. The field, acting perpendicularly to the flow distributes the molecules (or particles) of a given component non-uniformly across the channel. The average velocity at which this component is displaced along the channel depends on the particular distribution of its molecules (or particles) within the various flow streamlines.

When the distribution profiles of the molecules of two components are different, these components are transported at different velocities along the channel by the non-uniform flow and can eventually become separated when they reach the channel outlet. This is the basis of the separation in FFF.

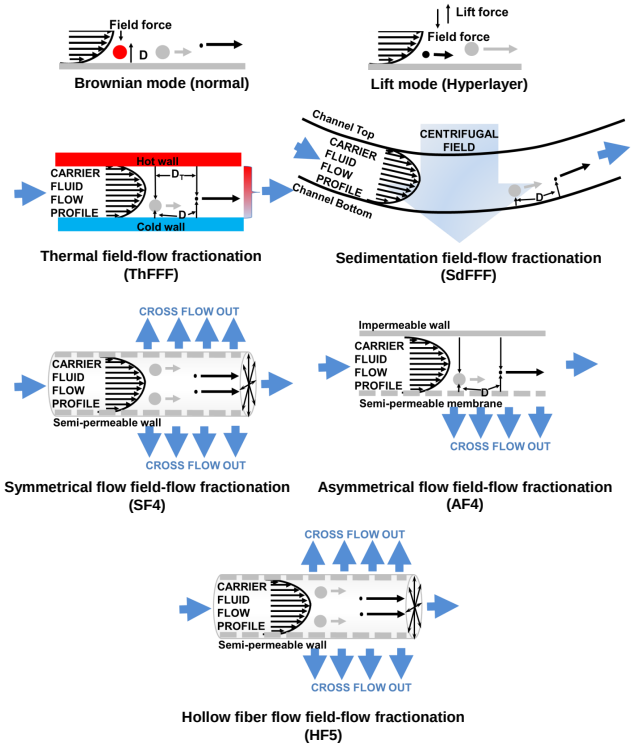

Fig. (1). Schemes of the FFF fractionation modes, ThFFF, SdFFF and Flow FFF systems.

There are several mechanisms that can lead to a non-uniform concentration distribution of the component in the field direction. For species that are relatively small (<1 μm), diffusion (*i.e.* Brownian motion) is the main mechanism that opposes the field force. This retention mode is named as "Brownian mode" (or normal mode). In this mode, a component is characterized from its mean sojourn time in the channel, the retention time t_R, by relating it to a dimensionless parameter, λ, which reflects the strength of its interaction with the applied field. λ, the basic FFF parameter, is expressed as:

$$\lambda = \frac{k_B T}{F w} = \frac{D}{U w} \qquad \textbf{(1)}$$

CHAPTER 6

Rheology of Recent Vegetal-Based Biopolymers

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Abstract: In this chapter, the rheology of polysaccharides extracted from cactus and agave plants is summarized. These vegetal sources of polysaccharides (*Opuntia* mucilage, *Opuntia* pectin, and agave fructans) were selected for their functional properties (thickening, gelling, or emulsifying) and their bioactive role in the the prevention and/or treatment of disease. The source, production, extraction processes, structure, and functional properties (related to conformation) of these polysaccharides are briefly described before the rheology of these biopolymers is discussed, and recommended uses are suggested.

Keywords: Agave plant, Carreau model, Characteristic time, Cross model, Fructans, Intrinsic viscosity, Loss modulus, Mucilage, Neolevans, Newtonian, Nopal, *Opuntia*, Pectin, Rheology, Shear rate, Shear-thinning, Storage modulus, Viscoelasticity, Viscosity, Zero shear viscosity.

INTRODUCTION

Biopolymers are polysaccharides and proteins with diverse applications in the food and pharmaceutical industries. These biomacromolecules are often referred to as hydrocolloids [1] and are added to perform functions including thickening and gelling of processed foods. Generally, polysaccharides are able to form viscous solutions or three-dimensional gel structures at low concentration, considerably modifying the rheology of the aqueous systems to which they are added.

To date, the vegetal-based biopolymers that predominate in industrial applications are obtained from various sources and include cellulose and its derivatives (major structural polysaccharides in the cell walls of higher plants): microcrystalline cellulose, carboxymethylcellulose, methylcellulose; starch (corn, potato, sweet

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potato, wheat, sago palm, tapioca, rice, banana, soybean, arrowroot, bracken, lotus, cassava, and waxy maize); hemicelluloses, including, mannans and galactomannans (guar and locust or carob bean and taro), xyloglucans, glucomannans, arabinoxylans, β-D-glucans, and arabinogalactan; pectins (citrus, apple, and sugar beet); exudate gums (arabic, tragacanth, karaya, and ghatti) and seaweed gums (alginate, carrageenans, agar) [1 - 3]. Some of these biopolymers have been modified physically or chemically to enhance their functional properties, and their rheology has been extensively studied and reported elsewhere [4 - 7].

Vegetal sources provide an abundant supply of biopolymers. In this chapter, we discussed the rheology of more recent sources of vegetal polysaccharides in detail that are consumed for their health benefits, such as *Opuntia* mucilage and pectins and agave fructans, which have received less attention.

Opuntia Polysaccharides

Sources

Members of the genus *Opuntia* from the Cactaceae family characteristically produce polysaccharide mucilages and pectins. The Cactaceae family is reported to consist of nearly 1,500 species from approximately 130 genera [8]. Cactus pear, commonly known as prickly pear and produced by the perennial *Opuntia* cactus, is well adapted to arid and semiarid zones, where water for cultivation can be limited. This genus is endemic to the Americas and is found in many countries (Argentina, Bolivia, Brazil, Chile, Egypt, Eritrea, Ethiopia, Greece, India, Israel, Italy, Mexico, Peru, Morocco, Spain, Tunisia, South Africa, and the state of Texas (USA)) [8, 9].

The cactus (*Opuntia ficus-indica, Ofi*) that produces the prickly pear is native to Mexico and can be consumed as a fruit or a green vegetable, and young leaves can be used in salad dishes. Cacti produce edible stems known as pads, phylloclades, or cladodes; synonyms are “nopales” or “pencas.” Nopal is consumed as a vegetable at different stages but primarily as fresh, tender nopal stems called “nopalitos,” which correspond to the first stage of development (less than 32 days of growth) [9, 10].

Many species of *Opuntia* cacti also produce a desirable fruit known as cactus pear, a many-seeded berry with a thick peel that encloses a flavorful pulp [11]. For example, *Opuntia ficus-indica Mill* is cultivated in Chile for fruit production [12].

Chemical characterizations of nopal showed that the presence of carbohydrates, dietary fiber, proteins, glycoproteins, vitamins, and minerals [8 - 10, 13]. Recently, non-nutritive components of cactus pear have been shown to be potentially active antioxidant phytochemicals. The composition of phenolic acids and flavonoids from *Opuntia* spp. have been reported in cladodes [10] and in cactus pears, including conjugated flavonoids such as quercetin, kaempferol, isorhamnetin, ascorbic acid, carotenoids, phenolic acids, and flavonoids [11]. Antioxidants are recognized protective against chronic disease by preventing the formation of free radicals. Sugars and acids are primarily responsible for the flavor of fruits, and the sugar:acid ratio is often used in harvest and quality indices of different fruit commodities [8].

Mucilage Production

Cladodes secrete a slimy fluid known as mucilage, which can also be found inside cladodes, fruit or fruit peel. In general, to obtain the mucilage, precipitation by alcohol (ethanol or 2-propanol) is performed after water extraction (homogenization of the cactus pads with water). However, very low yields are achieved (1-1.5% fresh weight, 19-22% dry weight) from fruit peel or pads [13].

A simple laboratory-scale extraction method was reported, where diced cladodes were blended in water, filtered, and centrifuged repeatedly; the extract was freeze-dried and resuspended in water, and trichloroacetic acid (TCA) was added before the mixture was left to stand prior to further centrifugation. The precipitate was resuspended and recentrifuged. The TCA-treated supernatant was dialyzed against distilled water for 24 h, precipitated with absolute ethanol and centrifuged to yield 0.7 g/kg of cladodes. Before extraction, the cladodes were boiled in 80% ethanol to inactivate enzymes [14].

Another method of extracting mucilage involves macerating the stems into a pulp and then centrifuging, decanting, and precipitating with acetone (1:2). The precipitate is then washed with isopropyl alcohol (1:1) and dried [15]. Alternatively, petroleum ether can be used to degrease the cladodes, and the pads are then macerated in deionized water and filtered under vacuum. The filtered product is directly freeze-dried or purified by ultrafiltration to eliminate salts and compounds of low molecular weight (Mw) [16].

The mucilage from peeled fruits is blended with water and filtered. The filtrate is centrifuged, and the supernatant is concentrated (vacuum) and dialyzed against distilled water (48 h). The resulting solution is concentrated under reduced pressure and poured into ethanol (1:5 v/v), and the resulting precipitate is separated by centrifugation, dissolved in distilled water, and freeze-dried [12].

CHAPTER 7

Gels of Ferulated Arabinoxylans: Rheology, Structural Parameters and Microstructure

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Abstract: One of the major by-products of bioethanol production is distillers dried grains with solubles (DDGS). Maize is one of the main sources for the production of this biofuel. In this way, dietary fiber represents the principal fraction of DDGS, which could be a potential source of added-value biomolecules such as ferulated arabinoxylans (AX). In this chapter, ferulated arabinoxylans extracted from DDGS (DDGAX) were gelled and the gels were studied in terms of rheology, structural parameters and microstructural characteristics. The DDGAX formed gels at 2% (w/v) induced by laccase. The mechanical spectrum and strain sweep of DDGAX gel presented the typical behavior of a solid-like material. The gels swelling ratio (43 g water/g DDGAX) suggested the formation of a compact polymeric network which decreased the water uptake of the gel. DDGAX gels presented an average mesh size value of 96 nm. The surface of the gels was analyzed by scanning electron microscopy revealing a heterogeneous microstructure resembling an imperfect honeycomb. These results indicate that ferulated arabinoxylans from DDGS form elastic and macroporous gels, presenting a microstructure with irregular pore sizes.

Keywords: Arabinoxylan gels, Ferulic acid, Gelling capability, Microstructure, Structural properties, Swelling.

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INTRODUCTION

One of the major by-products of the bioethanol industry is the distillers dried grains with soluble (DDGS). Because of the high amounts of DDGS generated during the process, new alternatives for the use of this by-product are needed.

Since dietary fiber represents a large fraction of the DDGS composition, DDGS is becoming a potential source of added-value biomolecules as arabinoxylans (AX). AX, also called pentosans, are non-starch polysaccharides present in various tissues of cereal grains. The structure of AX consists of linear (1-4)-β -D -xylopyranosyl chains to which α-L-arabinofuranosyl units are attached at the O-2 and/or O-3 positions [1]. Some arabinose residues are ester-linked on O-5 position to ferulic acid (3-methoxy-4-hydroxycinnamic acid) [2].

Hydrogels are hydrophilic polymer networks that are capable of imbibing large amounts of water without dissolution [3, 4]. In this way, ferulated AX can form gels by covalent cross-linking through dimerization of ferulic acid (FA) under oxidative conditions (*e.g.* use of free radical generating agents as laccase and peroxidase/H_2O_2) [5, 6]. Covalently cross-linked AX gels present important hydration properties, have absence of pH or electrolyte susceptibility and exhibit no syneresis after long periods of storage [7]. These gels have several potential applications, ranging from the food to pharmaceutical industries. Potential applications of AX gels as matrices for controlled release of biomolecules [8 - 10, 16] and cells [11] have been previously reported.

The gelling capability and gel properties of AX depend on their structural characteristics (FA content, A/X ratio, molecular weight) [12]. Gels with different rheological and structural characteristics can be obtained by modifying the source of the polysaccharide [13]. The structural parameters of nejayote, wheat and maize bran AX gels have been recently reported [5, 6, 13, 14]. In this chapter, DDGAX gels were characterized in terms of rheological characteristics, equilibrium swelling degree and microstructure. Knowledge of the equilibrium swelling degree allowed the calculation of gel structural parameters like molecular weight between cross-links (M_c), mesh size (ξ), and cross-linking density (ρ_c).

EXPERIMENTAL

Materials

AX from distillers dried grains with solubles (DDGAX) were obtained as previously reported and presented 69% dry basis (d.b.) of pure AX, a ferulic acid content of 6.05 μg/mg polysaccharide, a protein content of 8.2%, an A/X ratio of

1.08 and a molecular weight of 209 kDa [15]. Laccase (benzenediol:oxygen oxidoreductase, E.C.1.10.3.2) from *Trametes versicolor* and other chemical products were purchased from Sigma Co. (St. Louis, MO, US).

METHODS

Amino Acids Profile

The amino acid profile was obtained by HPLC according to the method reported by Vásquez-Ortiz *et al.*, 1995 [24]. The samples were hydrolyzed using 6 M HCl at 150 ºC for 6 h. After cooling, the hydrolysates were buffered to pH 2.2 prior to analysis by HPLC. The instrument used was an HPLC (Hewlett Packard Model 1100) equipped with Microsorb RP-C18 (10 cm x 4.6 mm ID) column and fluorescent detector.

Hydroxyproline and proline were determined by analyzing the mixture of 125 µL of sample with 500 µL of borate buffer and 250 µL of NBD-Cl. The mixture was heated at 60 ºC for 5 min and stopped with 50 µL of 1 M HCl. The amino acid standard mixture used was L-Amino Acids Standard (Sigma- Aldrich).

Gel Preparation

DDGAX solutions (2% w/v) were prepared in 0.05 M citrate-phosphate buffer, pH 5.5. Laccase (1.675 nkat per mg polysaccharide) was added to solutions as cross-linking agent for the formation of gels. Gels were allowed to develop for 1 h at 25 °C.

Rheology

Small amplitude oscillatory shear was used to follow the gelation process of DDGAX solutions. DDGAX solutions were mixed with laccase and immediately placed on the parallel-plate geometry (40 mm in diameter) of a strain controlled rheometer (Discovery HR-2 rheometer; TA Instruments, New Castle, DE, US). Exposed edges were recovered with silicone to prevent evaporation. The dynamic rheological parameters used to evaluate the gel network were the storage modulus (G'), loss modulus (G'') and crossover point (G'>G''). DDGAX gelation was followed at a frequency of 0.25 Hz and 5% strain. At the end of the network formation, a frequency sweep (0.01-10 Hz) and a strain sweep (0.02-20%) were carried out. The rheological measurements were performed in duplicate.

Swelling Experiment

A total of 2 mL of DDGAX solution at 2% (w/v) containing laccase was quickly transferred to a 5 mL tip-cutoff syringe (diameter 1.5 cm) and allowed to gel

CHAPTER 8

Sol and Gel Based on Polysaccharide: Characterization and Structure-properties Relationship

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Abstract: The objective of this chapter is to describe the main techniques adapted to characterize ionic or neutral polysaccharides, especially when they are water soluble. The steric exclusion chromatography (SEC), rheology and NMR spectroscopy are, in our opinion, the most important techniques to study the main characteristics of polysaccharides. The same techniques and approaches may also be valid for other water soluble biopolymers such as proteins and nucleic acids. The main characteristics of polyelectrolytes are recalled and it is shown that the ionic behavior of the polymers can be used to establish the nature of the polymer conformation (coil, helix). In this case, it is necessary to combine thermodynamic characteristics with optical rotary power (or differential scanning calorimetry DSC and circular dichroism CD) and molar mass determinationss. The semi-rigid characterization of the polysaccharide chains depending on their conformation is introduced. The persistence length L_p which characterizes the local stiffness of the chain determined by steric exclusion chromatography in application of the worm-like chain model and validated by molecular modelling. Rheology of polysaccharides is of great interest due to fundamental and applied point of views (especially in food, cosmetic or biomedical applications). The solutions even at low polymer concentration are often non Newtonian due to the stiffness of the polysaccharides which also depresses the critical overlap concentration. The main relationships relating viscosity to molar mass and/or polymer concentration are given; the influence of the shear rate for measurement is pointed out; and, the flow and dynamic measurements are described for sol systems. Originality of polysaccharides is that they may associate to form 3-D network, or physical network. This gel is controlled by the thermodynamic conditions and environmental conditions (ionic concentration, nature of ions, temperature, pH…). The main mechanisms of physical gelation are recalled and characterization of the sol-gel transition is introduced. The techniques to follow this transition as well as those for gel rheology are also introduced.

Keywords: Biopolymer, Characterization, Ionic selectivity, Polyelectrolyte, Polysaccharide, Rheology, Sol-gel transition, Steric exclusion chromatography,

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Viscosity, Water-soluble systems.

INTRODUCTION

In this chapter, we intend to expose the general properties of biopolymers and techniques used for their characterization. Especially, polysaccharides that I investigated for a long time will be discussed in this chapter [1, 2]. They are natural polymers or source of many derivatives which may be used in many industrial domains such as food, cosmetic, biomaterial, or pharmaceutics [3 - 6]. Natural polysaccharides produced by biosynthesis are based on sugar repeating units. They are often stereoregular and rich in –OH functional groups which form H-bonds. In stereoregular polysaccharides (carrageenans or bacterial polysaccharides like gellan or succinoglycan), intrachain H-bond networks often stabilize helical conformations. In addition, interchain H-bond networks stabilize physical gels or solid state materials with interesting physical properties (chitin and cellulose are semi-crystalline polysaccharides having good film forming characteristics) [7 - 9]. The stereoregularity is the basis of the cooperative helix-coil transition depending on the thermodynamic conditions (temperature, pH, ionic concentration, nature of the counterions…). As we will show later, helix –coil transition is often also related to the cooperative gel-sol transitions in aqueous polysaccharide systems [10].

The origins of polysaccharides are mainly plants, animals, fungi or bacteria. The polymers are neutral (as cellulose, amylose, curdlan, scleroglucan, agarose…) or ionic (xanthan, gellan, alginate, pectin…) becoming polyelectrolytes [11, 12]. The choice of the solvent composition used to prepare solutions and characterize polysaccharides is one of the most important step. For ionic systems, water is the best solvent, the solubility being promoted by electrostatic repulsions, and for neutral polymers, water often gives interacting systems (aggregates formation and low solubility) but dimethylsulfoxide or H-bond breakers are better. The problem of aggregation in such solutions, probably often induced after drying of the purified samples, is perhaps the most difficult problem to solve before going further in the polymer characterization.

After analysis of polyelectrolyte behavior, and indications on few techniques of polymer characterization (nuclear magnetic resonance NMR, steric exclusion chromatography SEC), we will focus on the rheological properties of these systems in sol and gel states, which are often the properties involved in applications such as thickening or gelling performances.

A-Polyelectrolyte Main Properties

When a macromolecule has ionic groups regularly fixed along its polymeric

chain, it is named as "polyelectrolyte". The parameter which controls its thermodynamic properties in solution is the charge parameter λ which is proportional to the linear charge density and introduced in the polyelectrolyte theories proposed by Katchalsky *et al.* [13] and later by Manning [14, 15]. It is expressed by the relationship:

$$\lambda = (\nu/h)\,(e^2/\,DkT) \qquad \textbf{(1)}$$

where ν is the number of ionic charges along a chain with a contour length h, e is the electronic charge, D is the dielectric constant taken as that of water (D = 78) and kT the Boltzmann term. The charge parameter λ is also written as λ = (ν/h)Q with Q the Bjerrum length, *i.e.* 7.2 Å at 25 °C in aqueous solution. λ is directly imposed by the distance b corresponding to the projection of two successive ionic sites on the axis of the chain (b is the length of a monomeric unit if each monomer has a dissociated ionic charge in the extended conformation). The distance b is reduced in case of a single chain helical conformation. The ionic sites may be $–COO^-$ (in pectins, alginates or hyaluronan), $-NH_3^+$ (for chitosan in acidic medium), and $-SO_3^-$ (in carrageenan). In the case of polyelectrolytes with $–COO^-$ and $-NH_3^+$ functions, the net charge (ν) depends strongly on the pH (in connection with the dissociation equilibrium) [16]. In addition, the electrostatic potential of the polyelectrolyte (or charged oligomers) increases progressively when the degree of polymerization increases and reaches a limit as soon as the number of charges (or degree of polymerization DP) of a charged block of ionized monomeric units becomes larger than around 15-20 when no change in the conformation occurs [17, 18]. At that time, thermodynamic properties become independent of the molecular weight (MW) but depend on the regularity of the charge distribution along the chains (example was discussed for blockwise distribution of galacturonic acid units along the chains in pectins) [19]. In addition, b is directly related to the conformation of the polymer; single or double chain helix formation implies a change of the length b and eventually of the molecular weight. This helical conformation often exists in stereoregular polysaccharides (or polypeptides and DNA). These single or double helices are stabilized by intra-chain and/or inter-chain H-bonded network formation [17, 20 - 22]. Following this, the study of electrostatic properties will help to characterize the conformation of these biopolymers in various experimental conditions (pH, ionic concentration, temperature) [10, 20].

A-1. Activity Coefficient of Counterions

Most useful information comes from the determinations of the free fraction of counterions to characterize a polyelectrolyte in the absence of external salt. Its theoretical value is directly related to the charge parameter and to the valency of

CHAPTER 9

Biopolymers from Mesquite Tree (*Prosopis* spp.)

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Abstract: The mesquite tree (*Prosopis* spp.) is a native plant species in arid zones that has been documented as a potential source of biopolymers, including polysaccharides, and more recently, proteins. The native flora of the Sonoran Desert offers considerable potential for the recovery of such compounds. Mesquite trees produce a water-soluble exudate known as mesquite gum (MG). Pods contain seeds with high levels of protein, and the endosperm yields a novel gum of the galactomannan family. MG and galactomannan (GM) are not yet considered food additives in the international market due to lack of FDA approval. However, structural, physicochemical and functional studies have shown that MG and GM are potential substitutes for commercial Arabic and guar gums, respectively. In addition, it was recently documented that mesquite seeds are a rich source of protein, with considerable quantities of essential amino acids. This chapter presents information about the structural and chemical characterization and applications of the biopolymers that can be obtained from *P. velutina.*

Keywords: Biopolymers, Galactomannan, Mesquite gum, Physico-chemical properties, Protein, Structural characteristics.

INTRODUCTION

Mesquite is the common name given to the trees of the *Prosopis* genus that are widespread in arid and semiarid regions of the world. The genus *Prosopis* grows mostly in the North America, South America, northern Africa and eastern Asia [1]. Mesquite is a multipurpose tree with the capacity to provide fuel, timber, fodder and edible pods, as well as biopolymers with characteristics similar to those currently used commercially.

The bark of *Prosopis* produces a viscous gum in the cavities of the xylem and

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phloem as a response to insect attack, wounding or weather conditions (temperature). Drought, age, and health of the tree can affect gum exudation. Mesquite gum is highly soluble in aqueous media (above 50% w/w) [2] and insoluble in alcohol or ether. The most important physical properties of the gum are its light color, high water solubility and viscosity, lack of odor and slightly astringent taste. The composition of gum collected in Sonora, Mexico, is 10% moisture, 2.6% ash, 0.35% tannin, 0.57% total nitrogen, 3.7% protein, and 96% total sugars, which include 71% arabinose (Ara), 26% galactose (Gal) and 3% uronic acids (UAc) [3, 4]. The intrinsic viscosity of the mesquite gum was calculated to be $[\eta] = 1.47 \times 10^{-2}\ Mw^{0.50}$ ($ml.g^{-1}$) [5]. This composition may vary with the species and variety of mesquite.

The mesquite tree produces copious pods. Its productivity depends on the age of the tree and soil characteristics. Pods are manually collected by rural families for use as feed for small livestock. Pods are composed of an external fibrous exocarp (56 wt%) that surrounds the mesocarp or pulp (composed mainly of sugars); the endocarp (35 wt%), is fibrous but much harder than the exocarp. Inside the endocarp are seeds (9 wt%) with 0.04 g average weight, 0.99 cm length, 0.56 cm in width and 0.22 cm in thickness. The endosperm of mesquite seeds develops alongside the germ (cotyledon) and completely envelops it. The endosperm itself is protected by a seed coat. The hard compact endosperm contains galactomannans that can reach 88% of its weight. The *P. pallida* seed cotyledon is 65% protein [6], while cotyledons from *P. velutina* are 32% protein. This protein fraction is well-balanced in essential amino acids [7]. Therefore, the use of mesquite biopolymers should be considered a potential source of valuable commodities and rural employment in arid regions.

Structural studies are a basic requirement for understanding the functional properties of biopolymers such as polysaccharides and proteins. The physicochemical characteristics of these macromolecules are defined by molecular weight; monosaccharide composition and sequence; conformation, configuration and the position of glycoside linkages; particle size; solubility; size distribution; rheological behavior; gelling properties; and the surface and interfacial properties that are directly related to the structure. On the other hand, the chemical composition and molecular structure of biopolymers often depend on the source, extraction methods and any further processing conditions. The physicochemical and structural features of biopolymers obtained from mesquite, as well as their applications, are discussed throughout this chapter.

Mesquite Gum (Exudate from *Prosopis)*

Mesquite gum is a polysaccharide exudate obtained from the bark of *Prosopis*

spp. trees (Fig. **1**). In rural areas of Sonora, Mexico, mesquite gum is collected mostly from wild *P. velutina* trees and is used in small amounts in various domestic applications, folk medicine and as a chewable sweet [8].

Fig. (1). Exudate from a mesquite tree (*Prosopis velutina*).

Chemical Structure

The chemical composition of mesquite gum was reported many years ago [9]–[14]. Mesquite gum (from *P. juliflora*) is a highly branched polysaccharide with L-Ara, D-Gal and 4-O-methyl-D-glucuronic acid residues. More recently, it was demonstrated that after partial acid hydrolysis, the inner backbone chains of *P. juliflora* gum contain D-Gal residues linked by 1→3 and/or 1→6 linkages with β-D-configuration and residues of glucuronic acid and 4-O-methyl-D-glucuronic acid bound to D-Gal by α-D-(1→4) and β-D-(1→6) linkages as the end groups [15, 16].

In addition, it was found that the acid-labile peripheral chains are complex oligosaccharides containing L-Ara. Apart from the carbohydrate component, mesquite gum contains 4% protein, and tannins (0.35%) have also been detected [4, 17, 18]. An arabinogalactan (AG) from mesquite gum (*P. velutina*) contains a galactan-core polysaccharide composed of glucuronic acid and D-Gal. Branching occurs by the attachment of L-Ara oligomers of as many as ten sugar residues to

CHAPTER 10

Direct Measurement of Free Volume Properties in Polymeric Materials

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Abstract: There has been a long history of theoretical and experimental papers which are concerned with the development of the free volume concept to explain transport and diffusion in polymer systems. Several theoretical efforts also took place to clinch the mechanism of the glass transition on the basis of free volume concept. In spite of the strong presence of free volume concept in scientific arena, it carries ambiguity in terms of definition. Many consider free volume concept as nothing but a guide to discussion since it has no physical existence. Past papers on theory and experiments dealt with the development of free volume concept to explain transport and diffusion in polymers. Many models based on free volume concept, show a number of unsatisfactory features as they stand at present. However, a number of theoretical approaches emerged to achieve a unanimous definition of free volume concept. Since the last few decades, Positron Annihilation Lifetime Spectroscopy (PALS) has become a well-established experimental technique for gathering information on the microstructure of free volume in polymeric materials. A brief discussion is presented in this chapter on free volume models along with PALS effectiveness in investigating free volume properties.

Keywords: Free volume, Models, PALS technique, Polymer and Biopolymer.

INTRODUCTION

Free volume is practically related to the motion and thus is a few centuries old concept. The great Greek philosopher Epicurus (341-270BC), offered ‘free space’ [1] concept to explain movement of atoms and an acknowledgement is due to Epicurus' school, for this great service. Though it is known that the equilibrium and transport properties of fluids depend on the space available for molecular motion, but the lack of methods to measure free volume has limited the development of this perspective [2].

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EYRING [3] in '36-'37 defined liquid by stating that they treated it as liquid which was constituted with 'individual molecules' and 'each moving in an average potential field' because of the presence of respective neighbors. He called the 'partition function' equation of the liquid as the definition of free volume, V_f :

$$F_l = V_f \frac{(2\pi mkT)^{\frac{3}{2}}}{h^3} R_l V_i b_l \exp\left(\frac{\Delta E}{RT}\right) \quad \textbf{(1)}$$

Here ΔE is the energy of vaporization, R_l and $V_i b_l$ are the rotational and vibrational parts of the partition function of a molecule in the liquid respectively, m, k, h and T are the molecule mass, Boltzmann constant, Planck constant and absolute temperature respectively.

Yang Yu in his thesis defined that 'Free volume' is more a geometric and descriptive concept than energetic [4].

To define free volume, Vrentas & Vrentas [5] stated that free volume theory depends on the specific volume of a single component or a mixture, which is constituted with three different volumetric components, *i.e.* occupied volume, interstitial free volume and hole free volume. The free volume theory is popular among the researchers dealing with polymers. It is easy to understand many properties of polymer at a molecular level with the help of this theory when clubbed with quantum and statistical mechanical calculations. Inspite of development of several free volume theories, a simple expression of free volume (V_f) can be more suitable for applications, such as [6], total volume (V_t) minus the occupied Volume (V_0): $V_f = V_t - V_0$.

'Free volume' is related to the microscopic empty space existing among the molecules and is represented by V_f. The specific free volume is given by the relation $V_f = V - V_{occ}$ where V is the specific total volume in cm^3/g and V_{occ} is the specific occupied volume which can be defined as the van der Waals volume V_W*i.e.* the volume of the space occupied by the molecule. In normal thermal energies this molecular space remains impenetrable to other molecules and V_f stands for the total free volume. It can be classified into two groups, namely, 'excess free volume' which appears due to the zigzag orientation of free volume chains forming irregular shape in sub nanometer scale and mostly appears in the amorphous region of polymers causing a decrease of about 10% in the density of the material in comparison to the corresponding crystal and the 'interstitial free volume' which is due to the unoccupied spaces appearing between two regularly packed chains and exists in both amorphous and crystalline region of polymer material. The following Figs. (**1** and **2**) show the two kinds of free volumes in the material:

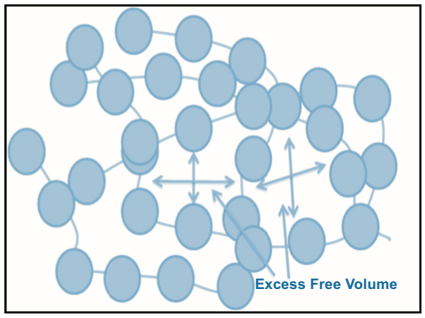

Fig. (1). Excess free volumes in amorphous regions.

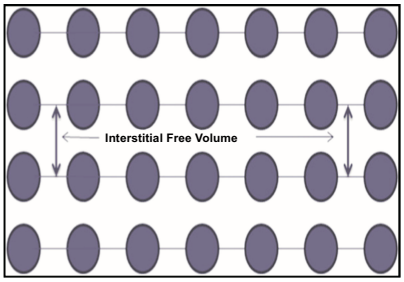

Fig. (2). Interstitial free volume in crystalline region.

These local free volumes are generally termed as "holes", thus the relation above can be rewritten in terms of hole free volume as $V_{fh} = V_f = V - V_{occ}$ and this V_{occ} involves both van der Waals volume and the interstitial free volume *i.e.* $V_{occ} = V_W + V_{fi.}$ The presence of hole free volume controls the diffusion of small molecules through molecular materials and also the molecular mobility [7, 8].

The occupied volume resembles approximately the crystalline volume and can be expressed as $V_{occ} \approx V_c \approx 1.45V_W$ [9]. The local free volumes or "holes" in the sub nanometer scale can be defined in terms of the average hole volume V_h and the specific hole number density, N_h' (in g^{-1}) by the expression, $V_f = V_h \times N_h'$.

The other important parameter for free volume concept is the free volume fraction which is defined as the ratio of the free volume to the total volume, *i.e.* $f = V_f / V$ and $y = 1\text{-}f$ = the corresponding occupied fraction.

Since there are several ways to define the free volume in polymer materials, the values reported for the free volume fraction, f, show a wide variation in the range from few % (percent) to about 20% [6].

However, Fujita [10] asserted that experimentally the specific volume of a given liquid, V (= N*v* ; where *v* is the liquid volume per molecule), is measurable where N is the total number of the molecules per gram of liquid, then Nv_c (where v_c is the

CHAPTER 11

Structural Analysis of Sulfated Polysaccharides

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Abstract: Sulfated polysaccharides are widely distributed in nature. The aim of this chapter is to give a brief description of methods of structural characterization of sulfated galactans, fucans, mannans, and arabinans from seaweeds, and sulfated polysaccharides rich in uronic acids (glycosaminoglycans and polysaccharides from green seaweeds), among other sulfated biopolymers.

These polysaccharides are heterogeneous with respect to chain length and sulfate content and must be purified to homogeneity before structural analysis is carried out.

Structural analysis of sulfated polysaccharides may be carried out by chemical methods: carbohydrate and sulfate content, monosaccharide composition, methylation/ ethylation and desulfation-methylation/ethylation, Smith degradation, *etc.*

Herein, the application of matrix-assisted laser-desorption ionization (MALDI) and electrospray ionization (ESI) mass spectrometry (MS), and nuclear magnetic resonance spectroscopy (NMR) in some of their wide variety of experimental methods is described.

^{1}H and ^{13}C NMR spectroscopy, together with 2D NMR techniques, provide very important information about sequence, interresidue linkage position and substitution pattern. MALDI-MS and ESI-MS have become important tools in the last years, however, they have limitations due to the labile nature of the sulfate group. In MALDI-MS, desorption difficulties with increasing molecular weight were also found. Thus, before application of mass spectrometry, depolymerization in controlled conditions is often required.

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Keywords: ESI-MS, Glycosaminoglycans, MALDI-MS, NMR spectroscopy, Sulfated arabinans, Sulfated fucans, Sulfated galactans, Sulfated polysaccharides.

INTRODUCTION

Sulfated polysaccharides are extensively distributed in nature organisms from seaweeds to mammals. Their presence in marine organisms has been associated with their adaptation to the high ionic strength characteristic of marine environment. This is thought to be the reason why they are present in seaweeds and marine plants, but they are not found in terrestrial plants and fresh water algae. They have also been found in marine invertebrates, like sea urchins, sea cucumbers, and ascidians. The high content of sulfate groups on their chains makes them polyanionic compounds of electrostatic-dependent intermolecular binding properties with relevant health/disease-related basic proteins or positively charged assemblies like basic amino acid clusters in virus particles. Overall, the specific bindings of sulfated polysaccharides with basic proteins (or domains in viral particles) and thus binding affinities in molecular interactions are found in accordance with the resultant pharmacological actions showing effectiveness of these glycans. The nature of these interactions is not a mere consequence of the net charge due to the degree of sulfation, but it is mostly driven by stereospecific features like monosaccharide composition, sulfation and glycosylation sites, anomericity, and conformational preference in their backbones.

In terms of total biomass, sulfated polysaccharides from marine organisms are more abundant than glycosaminoglycans. However, as the latter are present in the extracellular matrix of all animal cell surfaces, and they are important for human health, they have driven the highest research efforts and human and economic resources, resulting in very detailed knowledge of their complex structures. This was possible through the development of several advanced analytical methodologies [1 - 3].

The chemical structure of sulfated polysaccharides is very diverse, but they can be divided in the following major groups according to characteristics of their backbones.

1. Sulfated Galactans

Sulfated galactans from red seaweeds have linear chains of alternating 3-linked β-galactopyranosyl residues (A-units) and 4-linked α-galactopyranosyl residues (B-units). The A-units always belong to the D-series, whereas the B-units include residues of the D- or L-series, many times occurring as 3,6-anhydrogalacto-pyranosyl moieties [4]. They are classified in carrageenans and agarans according to whether the 4-linked residues belong to the D- or L-series, respectively. The

neutral, completely cyclized agaran is called agarose, commercially known as agar. Most of the other galactans have varied degrees of sulfation, as well as other substituents, as pyruvic acid ketal (occurring as 3-linked 4,6-*O*-(1′-carboxy) ethylidene-D-galactopyranose units), methoxyl groups and, sometimes, side chains, usually comprising single stubs of β-D-xylose. Fig. (**1A** and **1B**) show the structure of the commercially most important polysaccharides of this group.

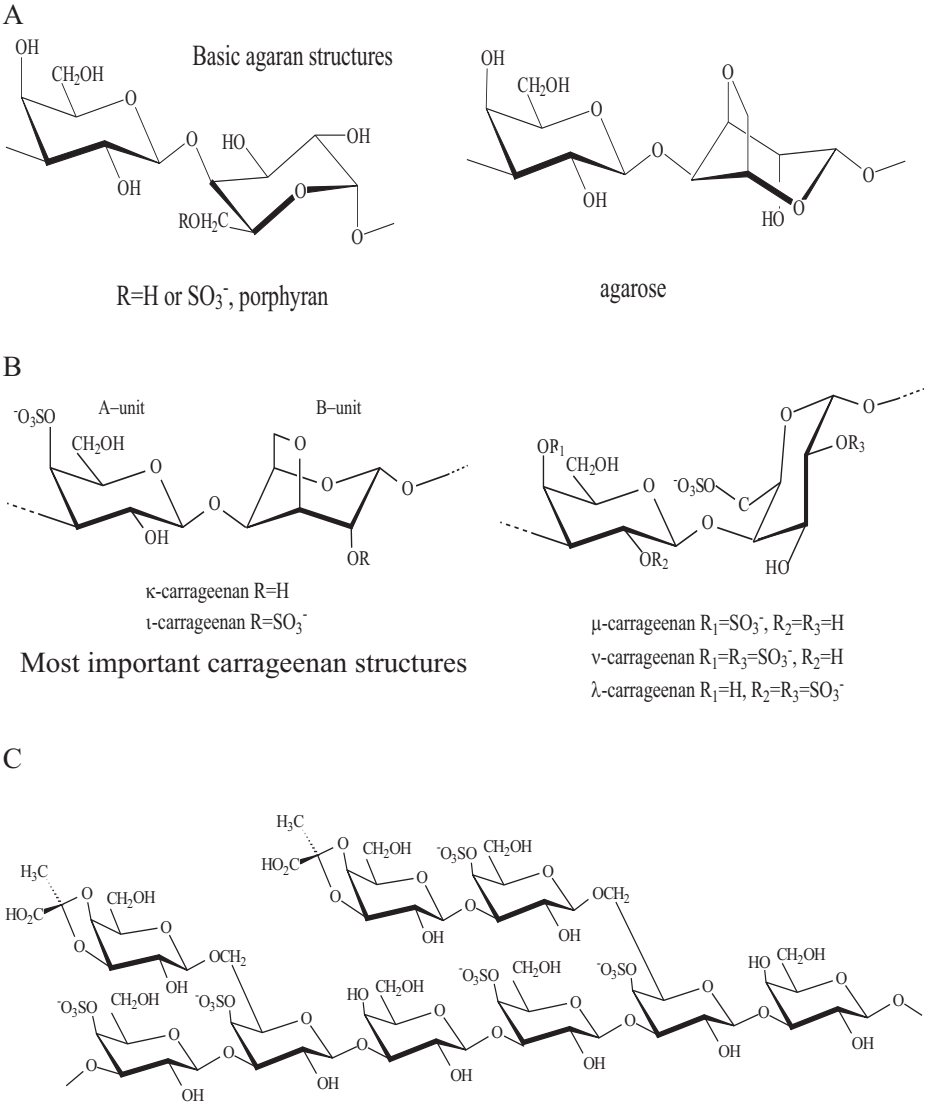

Fig. (1). Some sulfated galactan structures. A. Most important agaran structures. B. Major carrageenan structures. C. Galactans from *Codium* spp.

CHAPTER 12

Physicochemical, Antimicrobial and Mechanical Properties of Thermoplastic Materials Based on Biopolymers with Application in the Food Industry

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Abstract: Currently, there is great interest in the study of new ecological technologies searching a harmonious lifestyle with the environment. In this sense, numerous investigations on thermoplastic materials have been conducted as a significant alternative due to their promising applications. In this regard, thermoplastics materials from different biodegradable and edible polymer sources, which can be used in food packaging, films or coatings, have been evaluated as an alternative to replace synthetic materials to contribute to environmental pollution. The aim of this chapter was to study the physicochemical, mechanical and structural properties of biopolymer films for use in the food industry. In this chapter, different components used for the production of biodegradable and edible coatings: biopolymers, plasticizers, antimicrobials and antioxidants are reviewed.

Keywords: Biopolymers, Edible films, Food industry, Physicochemical and mechanical properties, Thermoplastic material.

INTRODUCTION

The current lifestyle has led the consumers change their eating habits. For this reason, the consumption of processed foods has increased noticeably in the last

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decades, and this has also increased the consumption and disposal of food packaging. This is how the need to reduce the environmental impact caused by these materials is highlighted. Therefore, the use of biopolymers-based thermoplastic materials have been proposed as an alternative to the use of synthetic materials made from the petroleum, since these are ecological and edible materials. Furthermore, these thermoplastic materials have improved food safety and quality, because they reduce the permeability to gases (O_2, CO_2, H_2O and ethylene), affecting the food spoilage. In addition, these biomaterials are an excellent vehicle for the incorporation of antimicrobial agents and natural antioxidants that allow to ensure the food safety. In the same way, the additives incorporated can improve the firmness, brightness and color of food, and even in processed foods.

In this chapter, we focus on the analysis of the different biopolymers used in the production of edible films such as the thermoplastic materials, as well as the physicochemical, mechanical, antimicrobial and structural properties of these edible plastics will be analyzed.

1- Biodegradable and Edible Films Based on Biopolymers

Most materials used for food packaging are petroleum-based polymers, due to their wide availability, low cost, great tensile strength and strain at break, as well as the oxygen barrier properties and heat sealability. However, these materials are not biodegradable, causing serious environmental problems. As a result, the demands have been directed towards the use of biodegradable materials obtained from agricultural products, such as the wastes from the food industry and from natural resources of low cost. Among the possible biopolymers used for the manufacture of films; carbohydrates, starches, pectic materials, vegetable and animal proteins are the most commonly used [1].

A film consists of a preformed thin layer of protective material formed for different components which vary depending on the functionality of the film. In several cases, it is applied on food products to reduce their exposure from the environment, as well as to establish barriers to moisture and oxygen, and solutes movement. They may also be fulfill the function of transport of several food additives, such as antimicrobials, antioxidants, antisoftening, micronutrients and bioactive components, which offer properties that can ensure or improve the food quality, safety and functionality [2 - 7].

Edible and biodegradable films have also been used to reduce the deleterious effects produced by processing conditions and food storage. This because the semipermeable barrier of the films can extend food lifetime, due to the reduction of moisture loss, solute migration, gases exchange, respiration rates and oxidative

reactions [8].

2- Film Components

The basic formulation of edible films includes a forming agent, which allows to obtain a three dimensional network or a matrix capable of self-supported, that at the same time preferably should be translucent and homogeneous. The components used can be classified into three categories: hydrocolloids (polysaccharides and proteins), lipids (fatty acids and waxes) and composites (hydrocolloids and lipids mixtures or combinations of components of the same group) [9, 10]. In this chapter the characteristics and properties of thermoplastic materials based on biopolymers will emphasized.

2.1- Polysaccharides

Polysaccharides have been the focus of research in recent decades, in order to be used as base material for the formation of biodegradable and edible films. Beyond their importance in contributing to the conservation of the environment and the possibility of extending the food shelf life, a remarkable reason of the use of polysaccharides is their low cost compared to the materials obtained from conventional sources as petroleum.

Polysaccharides are composed of monosaccharides linked by glycosidic bonds [11]. According to the monomers present in the molecules, they are classified as: *Homopolysaccharide* in the case of only one type of monosaccharide, and *Heteropolysaccharides* when the molecules are composed of more than one type of monosaccharides. On the other hand, there is another classification depending on their structure: linear structure (agar, methylcellulose, hydroxypropyl methyl cellulose, microcrystalline cellulose, inulin, carrageenan, pectin, gellan gum, carboxymethylcellulose, chitosan), branched structure (arabic gum, ghatti), or any combination, *e.g.* starches [11].

Polysaccharides most commonly used for the preparation of edible films or coatings are starch, alginate, pectin, algae extracts, gums, chitosan and different types of cellulose [12]. An important advantage of the systems formed by this type of component is their hydrophilic nature which provides a water vapor barrier, thus allowing the coatings avoid the moisture loss from the foods [8, 13].

The characteristics of the most important polysaccharides used for the preparation of thermoplastic materials to be used in the food industry are described below:

2.1.1- Starch

Currently, the use of starches is the focus of study in the food industry, because is

CHAPTER 13

Influence of Nanostructures in the Physicochemical Properties of Polysaccharide Based Biocomposites: Characterization and Applications

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Abstract: The design and engineering of innovative bionanocomposites for a wide variety of applications should be based on the understanding of the relationship between their nanoscale structure and their chemical and mechanical properties. The understanding of the chemistry and structure at the nanometric level imply an essential knowledge for future developments of novel materials based on polysaccharides and nanostructured materials toward several applications (for instance bioremediation, medical applications, biotechnology, food industry, *etc.*). The study of the physico-chemical characteristics of a material would imply not only its basic description but could be significantly relevant in the optimization of its synthesis toward the obtaining of tailored properties or the control of the material behavior in a particular application. Herein, we present the introduction to the most used techniques in the characterization of bionanocomposites, such as electron microscopies, FTIR, Raman spectroscopy, NMR, Rheology behavior, DSC, TGA, SAXS and SANS. The basics of these techniques and key approaches in the characterization of bionanocomposites are presented for the understanding of the relevance of the characterization step in a material evaluation.

Keywords: Biocomposite, DSC, FTIR, Nanostructure, NMR, Polysaccharide, Raman, Rheology, SAXS, TGA.

INTRODUCTION

In the past decade, the advances in the nanotechnology field increased. It has been pointed out that this rise was driven by the spread of instrumental techniques capable of analyzing the structure of a material in the nanometric scale, regarding

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both its physical and chemical properties. Tailored nanocomposite materials can be synthesized if the control of the heterogeneity of the structure on the nanometer scale is possible. From the molecular scale morphology plays a key role in achieving macroscopic molecular and supramolecular assemblies. The synthetic goal is to prepare multiphase micro- or nanoheterogeneous systems with desired and tunable properties. With this aim, a detailed multidisciplinary characterization is mandatory in order to establish the correlations among the composition, mechanical, viscoelastic, optical, and other macroscopic properties [1].

Regarding a material, that comprises a polymeric matrix and a nanostructure, including either nanoparticles or nanodomains (*i.e.* nanopores or nanometric structural levels of organization), the characterization often involves the separate study of the polymer and the nanostructure. With advances in the bionano-composites technology, it became more relevant to study the influence of nanostuctures in the physicochemical properties of the materials. When it comes to a biopolymer, the typical procedures of characterization are Nuclear Magnetic Resonance (NMR), Infrared (FTIR) and Raman spectroscopy for functional groups and carbon backbone analysis, size exclusion chromatography and light scattering methods for molecular weight determination, or rheological analysis for the study of their mechanical behavior, among others. Regarding nanoparticles, similar techniques for the study of their chemistry are often used, such as FTIR or Raman spectroscopy, also specific techniques focused on their nanosize are also employed. These techniques involve light scattering techniques, such as Static and Dynamic Light Scattering (SLS and DLS respectively), which are useful for size, distribution and polydispersity analysis. In addition, microscopy techniques, such as Transmission Electron Microscopy (TEM), Secondary Electron Microscopy (SEM) and Atomic Force Microscopy (AFM), are used for obtaining internal or topographical images, and Electrophoretic Light Scattering, for zeta potential measurements, among others.

Several of these techniques are used both for biopolymer and nanoparticle analysis and could bring information not only from each separate component of the bionanocomposite but could help to understand the interaction among them. In this chapter, we will try to cover most of these techniques from a point of view focused on obtaining information about the interaction of the biopolymer, mainly a polysaccharide, and the nanostructure. Herein we will present this subject in relation to Electron Microscopies, FTIR, Raman spectroscopy, NMR, Rheological methods, DSC, TGA, SAXS and SANS. The basics of these techniques and key approaches in the characterization of bionanocomposites are presented for the understanding of the potential of the characterization in a material assessment.

Electron Microscopies

With respect to physicochemical characterization, the analysis of a material or its components does not need to follow a mandatory order of techniques. Nevertheless, the techniques most likely to be performed in the first place are the Electron Microscopies (EM). The EMs bring information about morphology, topography, internal structure and other features that can be "observed" directly from an image. Therefore, they are not powerful techniques by themselves but they are powerful for the routine analysis and also when they are complemented with other techniques.

The significance of the EM in bionanocomposites science and technology arises principally from two causes. First, by using EM, information of the morphology of polymer matrix, the nanostructure and the contact interaction between them can be simultaneously assessed with micrometer to nanometer resolution. Second, electron microscopy allows studying the response of the structural details towards applied load, chemical and temperature exposure, time degradation, *etc.*, enabling the design of tailored materials. In addition, EM is the only technique, which provides direct evidence of intercalation and exfoliation of the nanostructure in the polymer matrix allowing straightforward quantification of morphological features of the polymer nanocomposites [2].

The main difference among EMs is the position of the detector which would lead to different sources of the image and, therefore, different information. Secondary Electron Microscopy (SEM) uses the sample holder as an anode towards which the electrons are accelerated. When the electron beam impacts the sample, an inelastic interaction can lead to the generation of secondary electrons that are expelled from the sample. In their path, they can reach a detector placed above the sample in a particular angle. Since the secondary electrons are only generated in outer layers of the sample, this image reflects the topography of the sample. If a new detector is placed near the lens, close to the electron beam path, a Backscattered Electron (BSE) image could be obtained. In this case, the detected electrons are the same electrons from the electron beam after elastically (or almost elastically) interacting with the sample nuclei and being backscattered to the detector. Since the probability of backscattering is higher with higher atomic numbers, the image presents a map of atomic number contrast. Thus, when inorganic nanoparticles became the part of a composite together with a biopolymer, they could be clearly detected as brighter islands surrounded by a darker polymer matrix [2].

From another point of view, a definitive elemental mapping could be obtained if an energy dispersive detector is placed in the sample chamber. From this

SECTION C. HYDRODYNAMIC PROPERTIES

CHAPTER 14

Intrinsic Viscosity of Strong Linear Polyelectrolytes in Solutions of Low Ionic Strength and Its Interpretation

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Abstract: Evaluation of reliable experimental quantities characterizing the isolated strong polyelectrolyte chain in solution at low ionic strengths is a challenge for researchers working in polymer science and biophysics. A simple method for estimating the intrinsic viscosity as initial slopes of the $\ln\eta_r$ and η_{sp} *vs. c* dependencies is discussed. Using several examples, we have demonstrated the adequacy of this estimated intrinsic viscosity. The results determined in salt free water solutions for homologous series of sodium poly(styrene-4-sulfonate) in 30-fold range of molar mass, as well as for the polyions with the persistence length of the corresponding bare chains differing 30 times are discussed. The "apparent intrinsic viscosities" of poly(styrene-4-sulfonate) samples at non-zero polymer concentration in salt-free solutions are compared with the directly measured intrinsic viscosities at different ionic strengths.

Keywords: Dilute solutions of charged polymers, Extremely low ionic strength, Intrinsic viscosity, Molecular hydrodynamic methods, Polyelectrolytes.

INTRODUCTION

Polyelectrolytes are polymers bearing various amounts of charges on their chains. Polyelectrolytes represent a broad class of polymers differing in charge density and topology of chains; they exhibit very different physical properties [1, 2] and they are the objects of permanent study due to their important role in living nature and in various technological processes [3 - 8]. The studies of conformational properties of linear polyelectrolyte are also of fundamental interest. Despite that, there is huge and continuing interest in polyelectrolytes as they remain one of the least understood states of soft matter. For linear highly charged polyelectrolyte,

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the question of the conformational status of charged chain at extremely low ionic strengths including the salt free solutions remains unclear till today, both theoretically and experimentally.

So far, according to the observation of Stevens and Kremer, polyelectrolytes are a typical example of a situation in which the experiments are carried out in semidilute regime, while the theories are best designed to dilute regime [5]. This utterance is pertinent until now. At the same time, it should be noted the disparity between the number of theoretical and experimental studies of highly charged macromolecules in solution, with the obvious predominance of the number of theoretical studies. This situation may be explained as follows. Due to the large size of charged macromolecules in dilute solutions at low ionic strengths, it is difficult to obtain characteristics that can be regarded as those of isolated macromolecules; experimental studies should be carried out at extremely low concentrations of polyions. Often, in these concentration ranges, the instruments used for measuring the corresponding experimental responses are not sensitive. Getting the adequate experimental characteristics of isolated polyions still remains a huge challenge for researchers till today.

One of the ways to characterize the isolated macromolecules of polyions in solution of extremely low ionic strength is the study of their viscous flow. The procedure for determination of intrinsic viscosity of polyelectrolytes in extremely low ionic strengths was worked up and reported earlier [9, 10].

This contribution describes: a) history and basic principles of the intrinsic viscosity in polymer science, b) the proposed method of estimating this characteristic for any kind of polymers, c) its application for the study of charged linear polymers, d) the ways of the interpretation of obtained results and comparison with the values obtained by conventional methods.

Hydrodynamic Methods and Essential Hydrodynamic Characteristics

The methods of molecular hydrodynamics (viscometry, velocity sedimentation, translational diffusion), as well as osmometry were actually the first experimental methods which enabled us to understand the nature of the polymeric material and realize that the basic structural unit of a polymer substance is a macromolecule [11 - 13].

Recently, valuable information about the conformations of the macromolecules were obtained also by scattering techniques, both X-ray and neutron scattering [14 - 18]. Nowadays the transmission and scanning electron as well as atomic force microscopy (TEM, SEM, and AFM, respectively) have become well-known, due to their unique potential to visualize individual macromolecules and to distinguish

between their topologies and conformations [19 - 22]. However, the study of macromolecules in solution, particularly of their homologous series, by the methods of molecular hydrodynamics, *especially the intrinsic viscosity measurements*, remains the indispensable base for understanding the conformational organization of the various types of macromolecules in solution. These methods have demand in molecular biophysics, in soft matter science, nano-, polymer- and colloid sciences [23 - 29].

The main methods of molecular hydrodynamics are analytical ultracentrifugation (velocity and equilibrium sedimentations), translational diffusion and viscous flow studies in dilute polymer solutions. Size exclusion chromatography (SEC) can be also included in this group of methods. These methods allow researchers to obtain a certain set of hydrodynamic characteristics, such as velocity sedimentation coefficient (s), translational diffusion coefficient (D), intrinsic viscosity ([η]) and some others. These characteristics are related to the molar mass and size of macromolecules in various ways. The molar mass can be determined within the framework of the methods of molecular hydrodynamics from the combination of the velocity sedimentation and translational diffusion data analysis, or from experiments on sedimentation equilibrium. The molar mass distribution of samples is determined usually from the SEC and/or from the velocity sedimentation analysis. Thus, the combination of hydrodynamic methods allows almost complete characterization of macromolecules, and determination of their gross conformation. The above techniques are applicable for studying neutral polymers or polyelectrolytes with the charge effects being virtually suppressed by the addition of low molecular mass salt. The principal content of this chapter is devoted to the discussion of characteristics of polyions in solutions with very low ionic strength (when polyelectrolyte effects are clearly observed). Molar mass of the charged macromolecules was determined by sedimentation-diffusion analysis of polyelectrolyte solutions with virtually vanishing polyelectrolyte effects. Needless to say that the knowledge of the molar mass of charged macromolecules is necessary for the interpretation of the results obtained in solutions of extremely low ionic strength. In this chapter, the paramount importance is paid to intrinsic viscosity as an important hydrodynamic characteristic.

The study of the viscosity of cellulose nitrate solutions by H. Staudinger led to the understanding of the structure of the polymer material and resulted in introduction of the term “macromolecule”. Thus, the important result of Staudinger’s work was the following statement [30]:

$$\lim_{(c\to 0)}\left(\eta_{\mathrm{sp}}/c\right) \equiv [\eta] \sim M, \qquad \textbf{(1)}$$

CHAPTER 15

Interrelation Between Polysaccharides and Different Surfactant Types

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Abstract: Interactions between polysaccharides and surfactants in aqueous solutions are the topic of this chapter. These components are often found in foods as well as in other systems like cosmetics, paints, detergents, textiles and many more. Therefore, investigation of the interactions between polysaccharides and surfactants has a great practical and fundamental interest. For the sake of clarity, we have divided the discussion into three sections: polysaccharides-nonionic surfactants; polysaccharides-cationic surfactants and polysaccharides-anionic surfactants. The behavior of polysaccharide-surfactant systems depends on many factors, which mainly include: 1) physical properties of both components, like molecular weight, degree of branching, number, and type of charge groups, backbone rigidity, 2) polysaccharide concentrations, 3) surfactant type; ionic or nonionic, polar head, chain length, and concentration. Environmental conditions like pH, ionic strength and presence of salt can partially screen electrostatic interactions, and there are also other factors needed to be considered when working with this type of mixtures. All these factors make it difficult to predict the behavior of polysaccharide-surfactant mixtures. However, in almost all cases, addition of surfactant modifies the behavior of the polysaccharide, regardless of polysaccharide and surfactant, but the modification is particular to the chemical nature of the polysaccharide and surfactant.

Keywords: Alginate, Biopolymers, Carboxymethylcellulose, Carrageenan, Chitosan, Electrostatic interactions, Emulsion, Hydrophobic interactions, Micelles, Polyelectrolyte, Polysaccharide, Surface tension, Surfactant.

INTRODUCTION

Foods are complex systems formed by a great number of components, including but not restricted to, sugars, proteins, polysaccharides, lipids, salts, and surfactants.

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These components may interact with each other in different ways depending on process conditions such as temperature and pH which make it difficult to control the shelf life and characteristics of the product such as texture and stability.

Surfactant is an abbreviation of surface active agent. A surfactant is a chemical compound with a tendency to adsorb at surfaces and interfaces. An interface is a boundary between two immiscible phases, and the term surface indicates that one of the phases is a gas, usually air [1]. The use of surface-active molecules in prepared foods is quite common. Low molecular weight surfactants, *e.g.* mono and diglycerides, phospholipids, are frequently used in combination with macromolecules like proteins and polysaccharides. In general, polysaccharides do not exhibit surface activity, but proteins can adsorb at interfaces and therefore it is important to know the interfacial properties and the dynamic behavior of all these molecules not only to understand the properties and behavior of complex systems but to develop products for specific purposes [2, 3].

Polysaccharides and surfactants are added to prepared foods basically to improve the stability of a food system. Polysaccharides are usually used to manage the rheological properties, while surfactants are used to handle the interfacial properties. In the case of polysaccharides, the main purpose is to get the desired viscoelastic properties. The interaction between polysaccharides and surfactants is studied in a model system (*i.e.* in aqueous solution), as foods are too complex to conduct fundamental studies. Therefore, most of our knowledge comes from a "simple" system. On the other hand, when polysaccharides and surfactants are used in combination, undesirable effects may occur, and the stability of the system can be affected. For example, incompatibility and formation of polysaccharide-surfactant complexes may eventually lead to phase separation. The interactions between polysaccharides and surfactants depend strongly on the properties of the former, on the type of the latter, on their respective concentrations and proportions in the mixture and on the environmental conditions of the aqueous medium, *e.g.* pH, ionic strength and temperature [4].

Surfactants can self-associate into micelles or micelle-like structures, and these aggregates can, in turn, interact with polymers present in the bulk phase. The concentration of surfactant when the interaction occurs is called the critical aggregation concentration (CAC). This concentration is lower than the critical micelle concentration (CMC) which is eventually reached by increasing the concentration of the surface-active molecule at a constant temperature. Polymers and surfactants can interact in different ways. Electrostatic, hydrophobic and dipolar interactions can be present depending on the nature of the polymer and the surfactant. Neutral polysaccharides can interact with anionic and cationic surfactants. However, the ways these interactions occur are different for each type

of surfactant. Charged polymers, *i.e.* polyelectrolytes, interact with oppositely charged surfactants by electrostatic attraction and when the surfactant and the polymer bear the same charge, interaction is still possible if the macromolecule is sufficiently hydrophobic. The behavior of surfactants is also different because anionic surfactants show stronger interaction than cationic ones of comparable chain length [5].

Interactions between surfactants and macromolecules are intensely investigated both for food and non-food applications. Most of the previous studies have been focused on protein and surfactant interaction while the interaction between polysaccharide and surfactant not currently approved for food use, has deserved less attention. Understanding the interactions between polysaccharides and surfactants can be crucial to obtain a given target product. In this chapter, the discussion is focused exclusively on the interaction between polysaccharides with nonionic, cationic and anionic surfactants regardless of the specific application, *i.e.* food or non-food. The chapter is expected to provide insight on the advancement and challenges of this interesting and complex subject.

POLYSACCHARIDES AND NONIONIC SURFACTANTS

Chitosan is one of the most studied polysaccharides and its interaction with different surfactants has been the object of numerous investigations. Mixtures of chitosan with nonionic surfactants like sorbitan monolaurate (Span 20), sorbitan monooleate (Span 80) and sorbitan trioleate (Span 85), have numerous applications in the food and cosmetic industries due to their ability to stabilize emulsions [6]. Although the interaction of chitosan with nonionic surfactants is weak, it is possible to prepare the emulsion, solution or cream with mixtures of various compositions of chitosan and sorbitan ester surfactants. However, some differences are observed depending on the chitosan-surfactant concentration and chain length of the surfactant. For example, the size of surfactant droplets dispersed in the continuous phase of chitosan is essentially the same (*ca.* 2.0 μm) in dilute (0.02 mol/dm^3) polysaccharide-surfactant solution. A ten-fold increase in the concentration of both components leads to higher droplet sizes, but the effect depends on the hydrophobicity of the surfactant; it is more dramatic for Span 80 in which aggregation of surfactant molecules leads to droplets approximately ten times larger than in dilute solutions [7]. On the other hand, confocal microscopy shows chitosan-surfactant aggregates formed by a chitosan-rich shell and a chitosan-poor core with highly ordered micrometric supramolecular structures resulting from high surfactant concentrations. These results indicate the affinity of chitosan, arising from its amphiphilic character to surfactants. This characteristic makes this polysaccharide capable of interacting at the interface of various colloidal systems like micelles, vesicles or emulsion droplets [6].

SECTION D. THEORETICAL MODELS

CHAPTER 16

Theoretical Models for Biopolymers

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Abstract: Theoretical models play a very important role in experimental practice and are essential for the development of current science and technology. They have been accessible for many years, but their development in recent decades, in parallel with the improvements in computation, has allowed the emergence of an important number of methodologies to complement experimental measurements. In this chapter, we present a brief overview of these methodologies. Among them, bead modeling has put a significant step forward. For rigid molecules, programs like those contained in the HYDRO suite have become very popular tools. For flexible molecules, computer simulation of their behavior in solution, either with a detailed atomic description (molecular dynamics) or based on coarse grain models for longer time scales (Brownian dynamics), is now a usual tool with a variety of accessible programs for use by scientists. Finally, a few examples of recent applications of these methodologies for proteins and polysaccharides are included. With this, we seek to illustrate to non-specialized readers the possibilities available today and, hopefully, to encourage them to find ways to incorporate theoretical models to complement their work.

Keywords: Bead modeling, Brownian dynamics, Flexible models, HYDRO, Molecular dynamics, Monte Carlo, Polysaccharides, Proteins, Rigid models.

INTRODUCTION

There is a very interesting philosophical discussion about modeling [1] and its role in experimental practice and the development of present science and technology. According to Zeidler [2], even when theoretical models can be corroborated empirically, they cannot be treated as representations of real empirical systems. This discussion is not within the scope of this book, but we agree with the statement.

The choice of the right model must follow a rule which has been well described

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by Carroll [3]: "We must choose the model that is sufficiently accurate for our computational purposes, yet still simple enough that we have some understanding of what the model describes. Otherwise, the model is a black box, and we have no understanding of what it does, perhaps even no idea whether the answers it produces are physically reasonable". For us, theoretical models are those used to represent biomolecules and their processes. They usually have two components: a physical representation of the system and a mathematical description of the internal and external processes that are taking place. Both components can be thought of in very different time and length (size) scales, depending on the natural phenomenon that we are trying to describe.

At present, computing is the basic tool for modeling. Virtually any kind of physical representation can be shown graphically in a computer, and the mathematical description of even the simplest processes of nature in which biopolymers is implied would be nearly impossible without the help of a computer.

Defining What to Model

Biopolymers, like all molecules, are composed of atoms made of electrons, protons and neutrons that follow the rules that quantum chemistry tries to describe. But the characteristics that we are interested in studying are those which come from the fact that these macromolecules contain thousands or even millions of atoms, and, yet their global behavior can be described in terms of a few (from one to hundreds) elements. This declaration immediately has vital consequences in the time scale, because we are not interested in phenomena lasting picoseconds or a few nanoseconds. We want to study things that take from many nanoseconds to microseconds to happen.

Another general statement is about flexibility. We can find biopolymers in the whole range of flexibilities. If a macromolecule needs a large amount of energy to change its conformation, we can predict that the changes in native conditions are going to be very few. Even if changes in small regions take place, for us, its global size and shape are going to be the same all the time (in our time scale). We describe this situation as a rigid molecule. Good examples are globular proteins. On the contrary, if we can easily find the atoms of a molecule in different positions (respect to each other) at the temperatures that we are studying, and we observe that the global shape (and size) is changing, we can say that we are working with a flexible molecule. Examples of this situation are some long linear polysaccharides or denatured proteins. Of course, these are the limiting cases and in nature, we usually find the whole range of possibilities or even mixture of them. That is why, we also define the so-called semi-flexible molecules with

different types of flexibility, with worm-like chains (like a long DNA molecule) and those with segmental flexibility (like myosin or many immunoglobulins) being very significant for biopolymers.

Building Models

To build a model, we must define the level of detail in the structure that is going to be modeled. Let us assume that the smallest unit we are going to distinguish is an atom. From this starting point, we can build a model describing the relative positions of all atoms present in a biopolymer. In recent decades, the extraordinary development of X-ray crystallography, structural NMR and cryo-electron microscopy has prompted this possibility for many molecules. Two important limits appear at this point. The first is that not all biopolymers are rigid or suitable for crystallization, and in many cases the positions of part of the structure (or all of it) remain unknown, and the second is that the positions of the atoms are going to change in solution (the usual state of the macromolecule) unless we take the polymer as infinitely rigid. If we handle a model at this level of resolution, we have an atomistic model. Another option is to group (how this is done is another matter) the atoms of a certain region to form a single unit to model the molecule. In most cases this unit is represented by a sphere (or a bead), giving rise to a coarse grain model, which is a very useful option for biopolymers, as we show in this chapter.

Once the structure has been formed (in an atomistic or in a coarse grain model), the size and shape of the model is defined if the molecule is considered rigid. But if any degree of flexibility is included, then connectors that reproduce the dynamic behavior of the molecule must also be included. In this case, the model becomes much more complex and the number of parameters is greatly increased. In addition, a procedure to determine the number of conformations present in the systems in certain conditions must be included.

In many occasions, it is interesting to know not only the characteristics of a molecule in solution at a certain instant, but also the dynamics of a system in which the studied molecule is only a part. When that is the case, computer simulation methodologies of the dynamics of a sample must also be included. In the following pages, we shall try to get an overview of the current situation of all of these aspects.

RIGID MODELS

The size and shape of rigid macromolecules are the only factors which determine their hydrodynamic properties. Scientists have taken advantage of this situation to develop hydrodynamic theories and methods to gain knowledge of the molecular

characteristics from experimental measurements. Thus, measurements of overall conformational and hydrodynamic properties in dilute solution (sedimentation and diffusion coefficients, intrinsic viscosity, radius of gyration…) using techniques like analytical ultracentrifugation, viscometry or small angle X-ray scattering (SAXS) have been an important source to obtain the overall size and shape of rigid (and flexible) structures. Classical theoretical approaches were based on elementary "whole-body" models that involve the exact hydrodynamic equations linking hydrodynamic measurements (rotational and translational diffusion, intrinsic viscosity…) with the axial ratios describing the ellipsoidal shape, along with some improvements in solving the "hydration problem" [4]. This simple ellipsoid-based modeling is useful for providing an overall idea of the molecular shape, particularly for those systems where only a limited amount of data is available. The suite of ELLIPS algorithms [5, 6] facilitates the use of this approach.

Most rigid biomolecules cannot be well described by defined geometrical forms like spheres, cylinders or ellipsoids. Illustrative examples are oligosaccharides, like cyclodextrins, and proteins, like hemoglobin, porin, calmodulin or those formed by more than one polypeptide chain. In 1967, Bloomfield *et al.* [7] proposed to model such complex molecules with spherical elements as building blocks, based on an idea first proposed by Kirkwood for flexible entities [8]. This is the bead model concept, according to which the shape of a particle is represented by an array of spherical elements ("beads") from which different properties of the particle can be obtained [9] (see Fig. **1**). Thus, the calculation of hydrodynamic properties of rigid particles can be carried out using methods from fluid mechanics combined with a generalized form of Einstein's theory of Brownian motion. This so-called "hydrodynamic bead modeling" has been shown to be very useful for the peculiar shapes of biomolecules [10, 11]. The ultimate level of detail in a bead model is reached if each atom (usually each extended atom including hydrogen) in the molecule is represented by a bead. Another possibility is to replace each repeating unit (amino acids, nucleotides…) with a bead [12]. A clear advantage of the latter is that the number of elements in the model is much smaller than in bead-per-atom models, and therefore it can be applied to large molecules. Other bead modeling strategies are also possible. Thus, if the rigid macromolecule is regarded as a compact, globular particle with a definite contour, then there is an immediate possibility of building a bead model that consists of filling the volume enclosed by that contour with beads. This is the type of approach implemented in the AtoB procedure [13]. Another efficient strategy is to build a "shell model" in which the molecular surface is modeled by an array of small and tangent beads while ignoring all internal molecular features, since hydrodynamic friction takes place only at the surface [10]. In bead modeling, care must be taken with bead overlap, since it adds problems to the

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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