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REVIEW

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Biophysical functionality in polysaccharides: from Lego-blocks to nano-particles

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Abstract The objective of the paper is to show the very important biophysical concepts that have been developed with polysaccharides. In particular, an attempt will be made to relate "a posteriori" the fundamental aspects, both experimental and theoretical, with some industrial applications of polysaccharide-based materials. The overview of chain conformational aspects includes relationships between topological features and local dynamics, exemplified for some naturally occurring carbohydrate polymers. Thus, by using simulation techniques and computational studies, the physicochemical properties of aqueous solutions of polysaccharides are interpreted. The relevance of conformational disorder–order transitions, chain aggregation, and phase separation to the underlying role of the ionic contribution to these processes is discussed. We stress the importance of combining information from analysis of experimental data with that from statistical–thermodynamic models for understanding the conformation, size, and functional stability of industrially important polysaccharides. The peculiar properties of polysaccharides in industrial applications are summarized for the particularly important example of nanoparticles production, a field of growing relevance and scientific interest.

Keywords Polysaccharide biophysics · Conformation and dynamics · Solution properties · Chain aggregation and gelation · Nanostructures and nanoparticles

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Introduction

Quite often polysaccharides are neglected in general polymer textbooks, even though they have the greatest chemical and structural variety among the three major classes of biopolymers. However, it happens that even biopolymers have often been regarded by industry as a less practical and not very convenient application, banishing them as a scientific curiosity.

Polysaccharides are constructed primarily, but not exclusively, from five and six-carbon sugars. The number of different sugar units found in naturally occurring polysaccharides is approximately the same as the number of amino acids used to build proteins, but each sugar has four or five hydroxyl functional groups. The multiple hydroxyl functionality of the sugars leads to several linearly linked chains, in addition to the occurrence of comb-like and tree-like branching (Suggett 1975). Thus, if one includes naturally occurring chemically modified sugars, the polysaccharides have chemical and structural variability that is not found among the polynucleotides and polypeptides. Many of the past commercial uses of polysaccharides seem to have been confined to foods, where market forces have been highly relevant. Nowadays, scientific and industrial interest in polysaccharide materials has moved them to the forefront of biocompatible polymeric architecture. Indeed, functional versatility among the polysaccharides derives from a wide variety of properties that do not include globularly folded forms. In many cases the functionality is closely associated with the occurrence of randomly coiling polymeric character, that is, a propensity for the chain to move continuously through a vast range of shapes of nearly equal energy. These chain topologies can, however, be tuned under specific conditions to provide nanostructured cages with intriguing performances in bio-oriented applications.

Over the last two decades, the most striking results, in the authors' opinion are:

1. elucidation of the morphological organization of polysaccharide moieties in starch granules (Banks and Greenwood 1975; Calvert 1997; Waigh et al. 1997);
2. use of innovative techniques, for example AFM microscopy and single-molecule mechanical spectroscopy (Brant 1999; Rief et al. 1998; Marszalek et al. 1998); and
3. investigation of chain conformation and dynamics (Kajiwara and Miyamoto 1998; Letardi et al. 2001).

As a consequence of these experimental methods and of other literature results, studies on polysaccharides in both the solid and solution state have clearly proved the existence of direct correlation between macroscopic effects and details of topological chain features (Rao et al. 1998). Many examples can be easily quoted in solid state carbohydrate materials, among which we draw attention to relaxation dynamics at sub-Tg temperatures (Scandola et al. 1991) and to the correlation between chain structure and plasticization temperature (Bizot et al. 1997).

The paper provides an overview of the main concepts of the conformational and dynamic properties of polysaccharides, and results recently obtained in several laboratories. The combination of these theoretical and experimental results has contributed to better understanding of macroscopic and applicative properties of these biopolymers. In the following discussion, conformational features and basic definitions of chain dimensions are presented first, by summarizing relevant static conformational aspects of carbohydrate polymers. Comparisons will be made among the simplest linear polysaccharides that share the same glucose monomer units, e.g., amylose, and the counterpart rigid poly-glucose chain exemplified by cellulose. We then discuss interpretation of theoretical results of dynamic properties, currently measured by use of different kinds of relaxation spectroscopy, e.g., NMR ($T1$). The comprehensive picture emerging from this approach will hopefully be that all the physicochemical "behavior history" of polysaccharides can, in principle, be written according to few structural determinants. However, appropriate theoretical algorithms and experimental determinations are needed to persuasively give complete and coherent descriptions of these polysaccharides. Finally, the last part of the paper will report on advanced exploitation in applied fields, from food to cosmetics to pharmaceutics and biomedicine, with a correlation between the general presentation and the industrial target and potential.

Polysaccharide conformation and dynamics in solutions

Energetics and conformation of polysaccharides in solution

The regularity of the polymer primary structure could imply that chains may assume ordered helical conformations, of either single or multiple strands, both in the solid state and in solution. Knowledge both of polysaccharide chain structure, up to the three-dimensional molecular shape, and the interaction of the polymers with other molecular components, is essential, in order to understand their capability to form supramolecular structures (Fig. 1). Physical gels with specific rheological properties have important implications for controlling and upgrading performance in industrial applications. The rationale is that the physicochemical properties, which are at the basis of the industrial applications of polysaccharides, are directly related to fundamentals of the structure and/or conformation of the chain.

Biological macromolecules in the solid state often assume regular helical structures which can be represented by means of a few geometrical data and symmetry relationships. These helical structures originate from the stereoregularity of backbone monomers and are easily

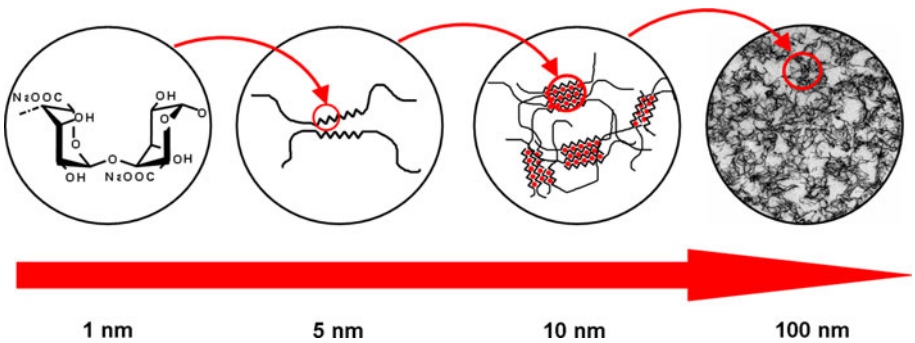

Fig. 1 Schematic representation of the supramolecular structure of the matrix of a polysaccharide hydrogel (here calcium alginate) on different scales, from the chemical units to the nanostructures. From *left* to *right*, in the frame of a nanotechnological vision: dimeric units; ordered segments; locally aggregated structures; the actual TEM micrograph of an alginate hydrogel (for details see Brun et al. 2011)

described, conceptually simple, and therefore used (all too often) as an idealized model for all the actual shapes. Elements of helical regularity are essential in the description of the structure of nucleic acids and polypeptides. Some biopolymers, e.g., globular proteins, almost completely preserve their structural regularity in solution. Such globular structures are not known in polysaccharides. Nonetheless, stereo-regular helical conformations have been proposed for many polysaccharides, microbial glycans in particular, and an excellent overview of their structures deduced from X-ray fiber diffraction studies has been published (Rao et al. 1998).

However, thermodynamic arguments suggest that a partially disordered state is an essential prerequisite for the stability of polymeric systems in solution. In these circumstances, realistic chain pictures of polysaccharides will not necessarily be generated by repetition of a single conformational state, which is usually identified by the minimum energy state found in the internal conformational energy calculations. Solution properties and statistical approaches to the conformational energy surfaces (Marchessault and Deslandes 1981; Brant 1982) suggest a more disordered solution conformation than the chain structures with the helical regularity deduced from X-ray fiber diffraction studies. Thus, thermal fluctuations are, in general, sufficient to generate delocalized disorder, unless weak non-specific interactions, cooperative in nature, lead to self-organization of the chain up to long-range order. Several polysaccharides with different chain linkage and anomeric configuration have been studied experimentally to determine the extent to which the polymeric linkage structure and the nature of the monomeric unit are responsible for the preferred solvation and for the chain topology and dimensions (Straub and Brant 1980).

In recent years, significant progress has been made in the experimental and theoretical research tools needed to study the conformational complexity of carbohydrates in solution, for example X-ray and neutron scattering techniques (SAXS and SANS), atomic force microscopy (AFM), high-resolution NMR spectroscopy and relaxation techniques, and computational methods. All the experimental and computational methods unequivocally indicate the relevance of monomer structure and linkage and of environmental effects (e.g., solvent composition, pH and salt conditions, and temperature) to the topological shape and properties of carbohydrate solutes (Table 1). The

Table 1 Structural features, solution behavior, and characteristic dimensional properties of some polysaccharides

Structural features	Solution behavior	Typical polymer	Characteristic properties
Chain linkage	Stiff polymer, water insoluble	Cellulose β-glc-(1-4)-glc	$L_p = 16$ nm[a] $C\infty = 48.8$[a]
	Flexible polymer, water soluble	Dextran α-glc-(1-6)-glc	$C\infty = 1.8$[b]
		Pullulan α-glc-(1-4)-α-glc-(1-6)-glc	$C\infty = 2.4$[b]
Side chain	Linear polymer, water insoluble	Cellulose β-glc-(1-4)-glc	$L_{p\ exp} = 16$ nm[a] $C\infty = 48.8$[a]
		Curdlan β-glc-(1-3)-glc	–
	Branched polymer, water soluble	Scleroglucan β-glc-(1-3)-glc branched in C_6	$L_p = 180 \pm 30$ nm[c]
Non-sugar substituents	Gel with Ca^{++} and Mg^{++}	Deacetylated gellan	–
	Soluble with Ca^{++} and Mg^{++}	Native (acetylated) gellan	$C\infty = 17.9$[c]
Ionic groups (e.g., carboxyl)	Neutral, water insoluble	α-Galactan; β-Galactan; α-Glucan (amylose)	α: $C\infty = 200$[c] β: $C\infty = 25$[c] $L_p = 2.8$ nm[d] $C\infty = 5.6$[d]
	Ionic, pH dependence, water soluble	Pectic acid; CM-amylose	$L_p = 10$–15 nm[e] $L_p = 5.6$ nm[f] $C\infty = 9.2$[f]

The chain stiffness is indicated by the values of the persistence length (Lp) and the characteristic ratio ($C\infty$)

Data quoted from:

[a] Hoogendam et al. (1998)

[b] Burton and Brant (1983)

[c] Yalpani (1988)

[d] Jordan et al. (1978)

[e] Morris et al. (2008)

[f] Goebel and Brant (1970)

general problem of the solvent effect on conformational states and preferential solvation of oligo and polysaccharides has been tackled mainly to validate detailed molecular models which were developed to relate the structural characteristics of these macromolecules to their chemical, physical, and biological properties in solution (Brant 1976; Dwek 1996). Even recently, however, semi-empirical methods have been proved very useful and generally applicable to different chain linkages and monomer composition (Liu et al. 2002).

The concept of chain conformational disordering and dynamics in solution is associated with the existence of a multiplicity of different conformations with accessible energy and, moreover, topological differentiation. The above conformational variability of polymeric chains is implicitly recognizable by the great difficulty in crystallization and by the typical phenomenon of polymorphism. In particular, this discussion is relevant for ionic polysaccharides (see below) because, the transition from a more compact conformation (also an "ordered chain") to an extended coil conformation is usually associated with a net variation in the ionic charge density along the chain.

Polysaccharides generally dissolve only in strongly solvating media. The behavior of water is complicated: it is a good solvent for monomers and oligosaccharides inasmuch it is able to compete with the specific inter and intramolecular hydrogen bond network (Talon et al. 2004; Brady et al. 2010). In many cases it is the thermodynamic stability of the solid-state form which protects the solute molecules from being solubilized (Cesàro 1986). Nevertheless, some other strong solvents, for example dimethyl sulfoxide and 1,4-dioxane, are known to be good solvents for carbohydrate polymers. Many commercial applications of polysaccharides require compatibility with different solvents and solutes (organic solvents, salts, emulsifiers, plasticizers, enzymes, etc.), for example in pharmaceutical matrices, paints, and foods. In this field, solvent compatibility of some glycans has been improved and controlled by functionalization and derivatization in order to obtain a proper degree of substitution, which determines a wide range of compatibility properties.

At the molecular level, a variety of specific and non-specific solvent–solute interactions may occur in polysaccharide solutions; these may result in a change in the conformational shape, solubility, viscosity, and other hydrodynamic and thermodynamic properties. Hydrophilic interactions, for example hydrogen bonding and electrostatic interactions, are believed to affect the conformation of polysaccharides in solution, although the question increasingly being raised is the implication of patches of hydrophobic intermolecular interactions, especially for chain aggregations. One important feature is the surface that saccharide segments present to solvent molecules, which enables many favorable interactions. The water structuring in the solvation of polysaccharides also contributes to the stability of saccharides in solution, which may be altered by competition of other co-solutes or co-solvents which are able to modify the extent of hydrogen-bonded interactions among components.

Chain conformation by computational methods

The rationale for correct quantitative description of the shape and dynamics of polysaccharides in solution is based on three correlated factors:

1. primary structure (i.e., the chemical identity of the carbohydrate units polymerized in the chain);
2. intrinsic conformational features dictated by the rotational equilibria (often the major contributions are from rotations about the glycosidic linkages); and
3. thermodynamic interaction with other molecular species (mainly the solvent which determines, therefore, chain solubility).

Over the last 20 years or so, several approaches have been exploited to determine the three dimensional "relaxed" structure of polysaccharides. Although computer facilities and calculation speeds have grown exponentially, the most sophisticated techniques, for example quantum mechanical methods (ab-initio methods), have been shown not to be useful for dealing with the complexity of many-particle systems, in the same way as for macromolecular media. On the other hand, these techniques have been successfully applied to small molecules, for example mono and disaccharides, for predicting charge distribution, preferred conformations, and transitions among accessible conformations, thus providing background knowledge for more complicated systems.

The dominant features in the molecular topology and flexibility of disaccharides and higher oligosaccharides are those which arise from rotation about glycosidic linkages (Burton and Brant 1983). Although other conformational fluctuations contribute to the local dynamics of atoms, or groups of atoms, only glycosidic linkage rotations are able to dramatically change the conformational topology of oligomers at room temperature. The objective of conformational analysis is thus to evaluate the probability (i.e., the energy) of all mutual orientations of the two nearby monosaccharide units, as a function of rotations about glycosidic linkages, as defined by the dihedral angles φ and ψ. Therefore, it has been the custom in the past to compute the conformational energy as a function of dihedral angles φ and ψ only, by assuming the sugar ring to be rigid (rigid-residue model). The rigid-residue approach is still regarded as a useful starting point in the conformational analysis of polysaccharides, provided the coordinates of all the atoms

in the monomeric unit have been calculated by use of a suitable independent method (Ruggiero et al. 1995). More recently, all the internal coordinates are allowed to adjust at each increment of φ and ψ, relaxing the structure towards a local minimum (relaxed-residue model). Figure 2 presents the basic features of conformational energy maps calculated for three dimeric units, cellobiose, maltose, and isomaltose. Schematic structures and location of the virtual bonds connecting consecutive glycosidic oxygen atoms are also shown (for the details and background, see Brant 1976). The important region of φ and ψ rotations is that "colored" with energy variations of the order of a few kT, the thermal motion energy, because this may produce a large ensemble of accessible conformational states for the oligosaccharide. Even when the rotational motion is restricted to a small range of angular values, fluctuation of many glycosidic bonds is amplified along the chain backbone, as the molecular weight increases. The accumulation of even limited local rotations may produce very large topological variations in the case of polymeric chains, and, consequently, relevant changes of thermodynamic, hydrodynamic, and rheological properties. Other internal motions often make only small contributions to observable properties on a macromolecular scale (Brant and Christ 1990).

Computer-based polysaccharide chain models can be constructed from the conformational energy map of the dimeric units, as shown in Fig. 2. The Flory matrix (Mattice and Suter 1994) and Monte Carlo methods (Jordan et al. 1978) are commonly used to mimic the polymer chains in the pure amorphous state or in dilute solution (in the so-called "nearest-neighbors approximation"). Although the rigorous Flory approach gives analytical solutions to average chain characteristics, the numerical Monte Carlo methods are also popular nowadays for pictorial description of instantaneous chain trajectories. Figure 3 shows snapshots of two representative chain segments generated by using the disaccharide geometry and energetics reported in Fig. 2. Essentially, the Monte Carlo sample of chains reflects the range of conformations experienced by any single chain as a function of time or, equivalently, the range of conformations in a large sample of chemically identical polymer molecules at any instant in time. In one sense, the sample can be analyzed to depict the characteristics of individual chain conformations or to deduce the mean properties of the sample as a whole, corresponding to those in the equilibrium chain state.

Polysaccharide chain local dynamics

Within the multiplicity of structural and conformational features (for a seminal review see Brant 1999), rules of thumb for chain topologies were assigned early to schematically ordered (Rees 1977) or statistically averaged (Burton and Brant 1983) chains and more recently extended to dynamic properties (Perico et al. 1999) of simple homo and copolyglycans. Exploitation of the latter properties has been made possible by progress in FT-NMR techniques which are among the most valuable tools for studying conformations and dynamics of molecules in solution, by determining chemical shifts, coupling constants, NOEs and relaxation times (Dais 1995; Duus et al. 2000).

One of the major breakthroughs in computational results for local dynamics has been achieved by determination of the spectral density of ^{13}C–^{1}H motion in the nanosecond frequency range simulated for linear homopolysaccharides differing in glycosidic linkage position and stereochemistry. The theoretical model is based on a diffusive approach (Perico 1989), which takes into account polymer connectivity together with an accurate description of the accessible conformational space. This approach, designated optimized Rouse Zimm local dynamics (ORZLD) theory, has been improved and implemented for application to random coil polysaccharides in solution. By using ORZLD, the second-order orientational correlation time, τ_i, has been calculated for each bond in the chain model for the different chain linkages and for several chain lengths (Perico et al. 1999). As an example, the insets of Fig. 3 show the virtual bond dynamic patterns for oligomers of amylose and cellulose at different chain lengths. All the curves have an overall bell shape; the greatest slowing of the fluctuations, i.e., the largest τ_i value, is found to be near the central residue. Terminal residues are always "more free to move", irrespective of the particular chain geometry. In the cases of the $\alpha(1 \rightarrow 4)$glc chain characteristic periodic undulations are observed in the trend of τ_i with a periodicity of approximately 7 residues. Figure 3 shows that the dynamic range of the positional dependence of τ_i increases strongly with the length of the $\beta(1 \rightarrow 4)$glc chain. This effect is because the polymer in question is so stiff that Cn is increasing with $C{=}n$ in the range investigated (and Cn is far from the asymptotic limit $C\infty$). Note that the stronger the increase in Cn with n, the greater the dynamic range of the positional dependence of τ_i.

Thus, the local dynamics are very sensitive to the microstructure of the polysaccharide, which in turn determines the extension of the chain. The more extended the chain, as measured by Cn (Table 1), the longer the corresponding correlation times. This correspondence is clearly indicated by the linearity shown in Fig. 4, in which the average (experimentally measurable) second-order orientational correlation time, τ_i, is log–log plotted as a function of $\langle r^2 \rangle$, the square end–end distance for cellulose and amylose. It seems worth mentioning that a correlation

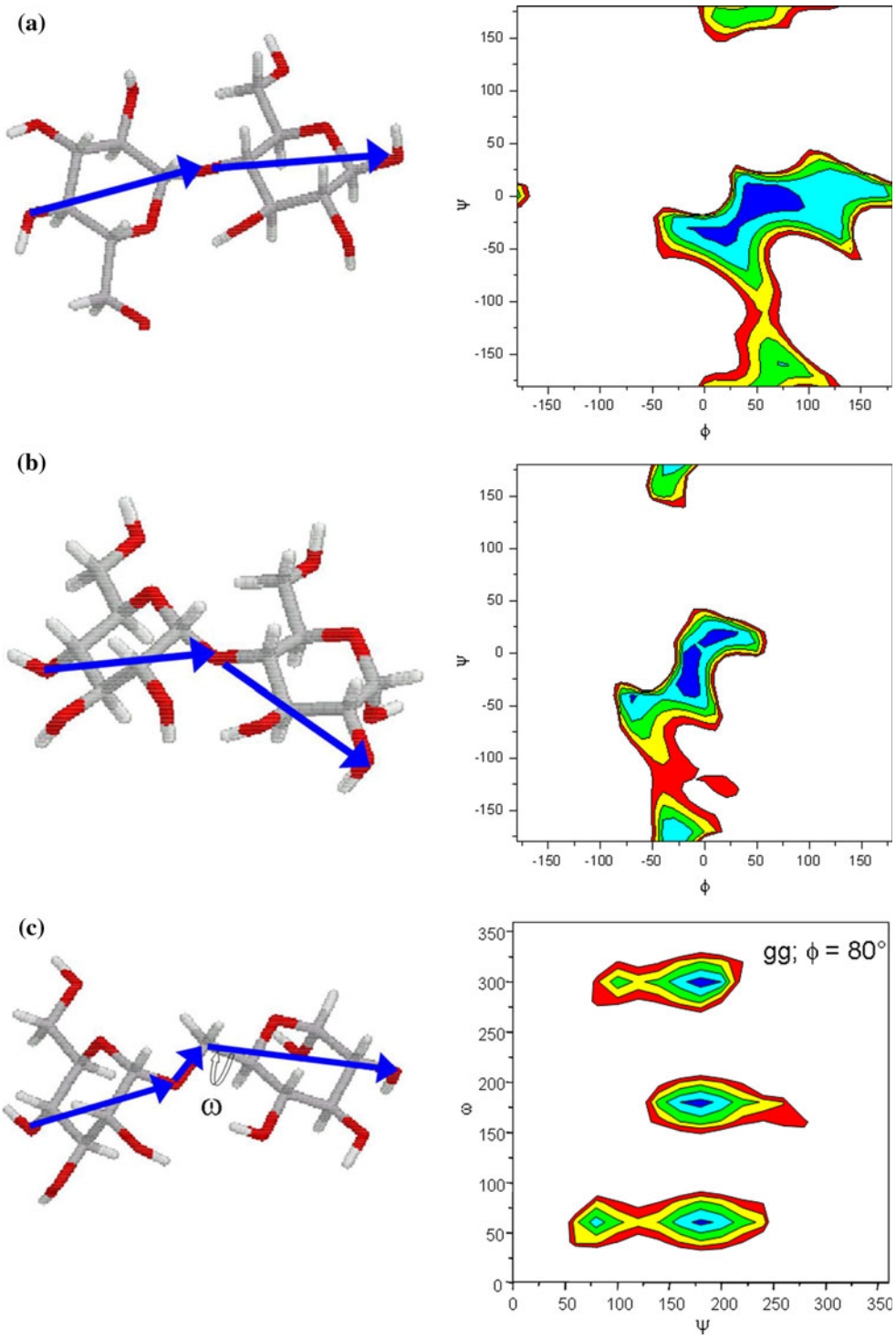

Fig. 2 Basic features of conformational energy maps (*right*) calculated for three disaccharide units: cellobiose **a**, maltose **b**, and isomaltose **c**. Schematic structures and location of the virtual bonds (*left*) connecting consecutive glycosidic oxygen atoms are also shown (for details and background, see Brant 1976). The highest probability region of φ and ψ rotations is that *colored blue*, with energy variations of the order of a few *kT* below the *white* region. Even if rotational motion is restricted to the low-energy regions, fluctuation of glycosidic bond orientations can be amplified along the chain backbone with many residues

between the "static" properties and the "dynamic" properties is intrinsic in the theory and somehow even "trivial": it is based on the fact that the matrices used contain the same conformational information. The local correlation times are sensitive to a correlation length, which increases with the stiffness of the chain. If the chain is longer than this correlation length, addition of further units does not affect the local dynamics of central units of the chain. In contrast, if the chain is shorter than the correlation length, the local dynamic pattern changes with the number of residues and τ_i increases with increasing n; in every case, the outer residues relax more quickly than the central ones. Therefore, this correlation length defines the meaning of local motion: the lower the stiffness of the chain, the shorter the

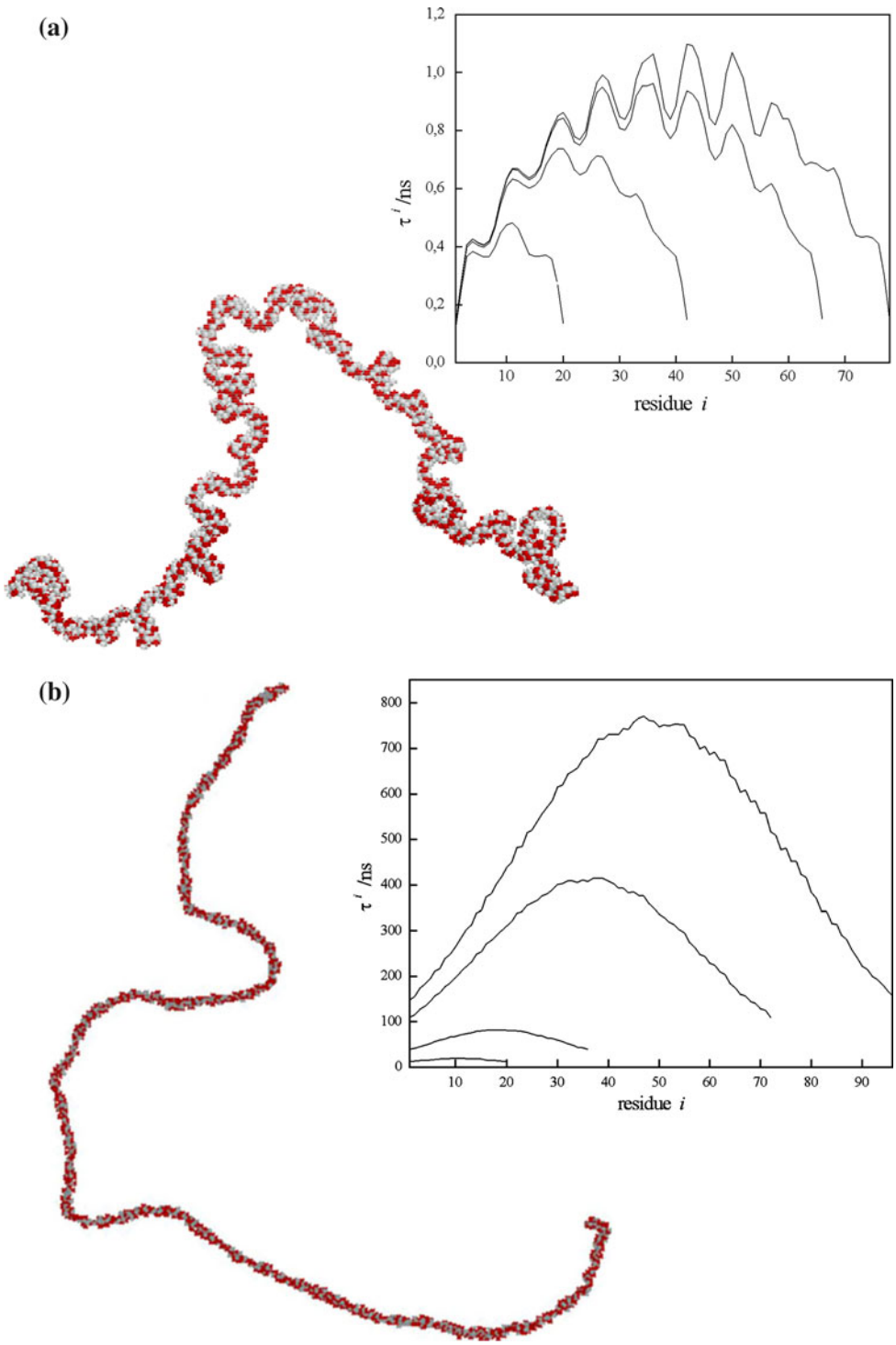

Fig. 3 Snapshots of the chain segments of amylose **a** and cellulose **b** generated by using the disaccharide geometry and energetics reported in Fig. 2. The *insets* show the virtual bond dynamic patterns for oligomers of amylose and cellulose, respectively, at the chain lengths indicated by the end of the *curves*

correlation length and the more local the motion. Although the theoretical basis was indicated above as trivial, the possibility of relating NMR relaxation data to the *Cn* static value and to the structure of the polymer is not trivial at all.

All the above findings can be generalized for other oligo and polysaccharide chains, even though the details depend on the model calculations and approximations. It seems necessary, however, to emphasize that the detailed structure of τ_i predicted by the calculations will never be experimentally observed in a chain longer than the appropriate correlation length or wavelength of the polymeric motions, because of the averaging effect among indistinguishable residues in the experiments, unless the sequence of residues is somehow "labeled" to become distinguishable.

A particularly interesting situation occurs in copolymeric glycans, for example hyaluronan, where the effect of

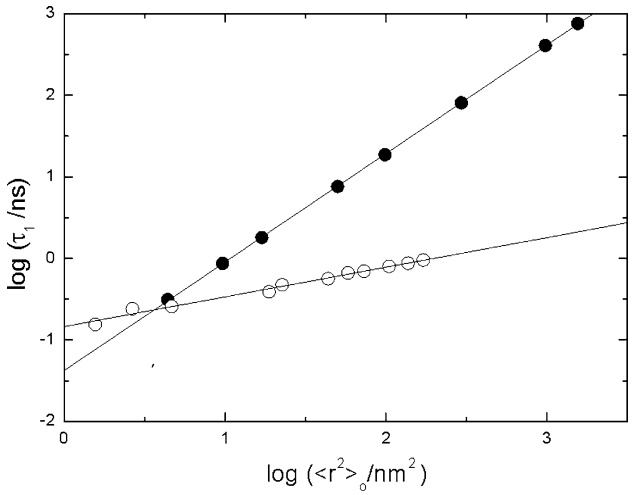

Fig. 4 Linear dependence (log–log) of the average correlation time, τ_i, on chain dimension, expressed by the end–end square distance for amylose (*open symbols*) and cellulose (*full symbols*)

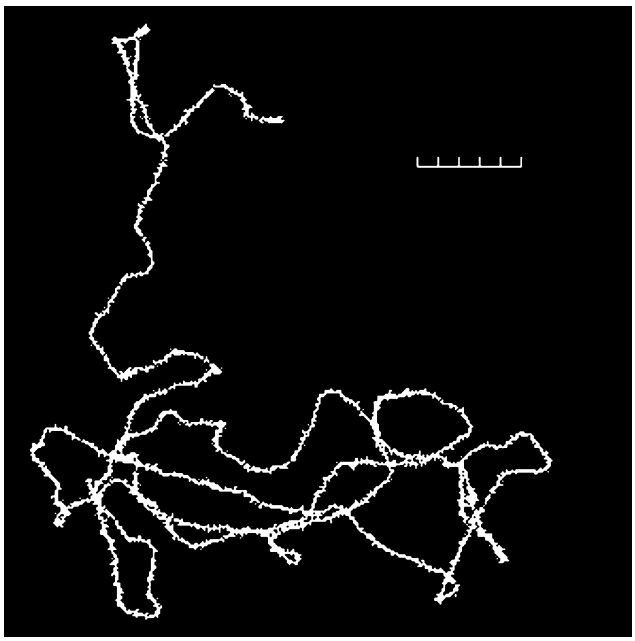

Fig. 5 Computer simulation (Monte Carlo snapshot) of a hyaluronan chain of 500 disaccharide residues. The conformational trajectory is based on the model validated by use of relaxation dynamic data, as described by Furlan et al. (2005). The *bar* denotes 10 nm

substituents on the backbone molecular conformation and dynamics is evident. It is claimed that the formation of interresidue hydrogen bonds between the carboxyl groups in the D-glucuronate residue (U) and the *N*-acetamido group in residue 2-deoxy-2-acetamido-D-glucose (A) can greatly modify local structure and mobility and, indirectly, change the solvation of the polymer segment. The constraints arising because of medium-range interactions, for example hydrogen bonds, and long-range interactions between the polar or charged groups must be included in any realistic model of hyaluronan polymers. For hyaluronan, almost every carbon chemical shift can be assigned up to the $(UA)_6$ oligomer and ^{13}C NMR relaxation can be monitored (Furlan et al. 2004). The rates of single exponential functions representing the decays, $R(C_z)$ and $R(C_{xy})$, are the inverse of the ^{13}C spin–lattice T_1 and spin–spin T_2 relaxation times, respectively, and are called relaxivities. Relaxivities are linear combinations of spectral densities that in turn are Fourier transforms of second-rank time autocorrelation functions (TCFs) of inter-nuclear distance vectors. Relaxivities are usually interpreted by finding a set of values in the modeling of second-rank TCFs that fits the largest number of experimental data at hand. To study possible networks of hydrogen bonds, electrostatic interactions, and solvation effects combined with short-range torsional potential occurring in hyaluronan oligomers, the most affordable numerical technique to obtain the statistics is the MD simulation algorithm. In the simulation, a deterministic trajectory of every configurational variable is acquired and canonical ensemble statistical averages necessary to the diffusion theory are estimated by time averaging along the trajectory. Thus, the combination of MD simulations with diffusion theory gives a microscopic description of NMR relaxation data for biopolymers, without any approximation concerning the separation of time scales (Tugarinov et al. 2001).

It must be stressed that most previous work focused on comparison of MD simulations and NMR data for hyaluronan and did not involve a quantitative and straight derivation of NMR data from MD trajectories; it did, however, include qualitative explanation of chemical shifts, fitted order data, and distance constraints. Given the good agreement between prediction and experiments on short hyaluronan oligomers, it has been possible to depict conformational and frictional properties, and average chain properties such as persistence length and radius of gyration, which compare well with literature data (Furlan et al. 2005). Figure 5 gives a snapshot of a hyaluronan chain of 500 disaccharide residues based on the model validated by use of relaxation dynamic data.

Polysaccharide conformational transitions

Whereas, overall, the random coil is the most probable spatial representation of high-molecular-weight polymers, the actual size and local shape may largely depend on the mutual interaction between solvent and polymer segments. This means that the presence of appreciable solvation of, or repulsion between, chain segments induces a coil expansion. The expansion factor for uncharged polymers mainly depends on interaction of the solvent with the polymer units, because solvation directly affects the statistical

weight of the isomeric states by favoring extended conformations.

Intrachain repulsion, which is strongly dependent on the ionic strength, occurs in polyelectrolytes. Because of the fixed charges of the segments, the intrinsic propensity of the chain is toward significant expansion. For polyelectrolytes, additional relevant properties are the effective charge density distribution, ρ, as defined by $\rho_q(\mathbf{r})$, and the "average" linear charge density ξ (and its distribution) along the chain axis as defined in the framework of polyelectrolyte counterion condensation (CC) theory (Manning 1979). At low ionic strength the repulsion between charged segments becomes very significant and the polymer coil adopts an expanded configuration. As the salt concentration is increased, the repulsion between charged segments decreases and at quite high ionic strength can be almost fully "screened" by ions in solution. Thus, when ionic expansion is depressed by an excess of added salt, the conformational features depend on the intrinsic covalent structure and short-range interactions. For many ionic polysaccharides the fixed charges are given by carboxylate groups and, therefore, the charge density (i.e., conformation) is pH-dependent. This concept can be quite important in practice for all the applications discussed later, because pH governs not only expansion of the polymer but also the inter-chain interactions. It may be worth mentioning that upon changing the pH, the actual charge density ξ of an ionic polysaccharide can span from very low ($\xi \ll 1$) to ξ values much larger than 1, thus undergoing CC effect (Cesàro et al. 1989). However, the rigid polyelectrolyte model does not seem to fit simple experimental data, for example the pH-titration of a polycarboxylic chain. In contrast, incorporation of conformational distribution and variability in the free energy of a polyelectrolyte system can enable quantitative fitting of experimental data, simply by summing the contributions of the ionic and non-ionic interactions. The high heuristic value of the CC polyelectrolytic theory lies in the simple theoretical formulation of the free energy term of electrostatic nature, making the evaluation of the temperature dependence (the entropic and enthalpic terms) also possible in a straightforward way. Thus, not only has the validity of the CC theory applied to a pH-dependent property been extended to cases in which a cooperative order–disorder transition or an aggregation take place (Cesàro et al. 1991), but also the related enthalpic contribution of ionic origin has been evaluated for several processes.

Dealing with biological polyelectrolytes, it is never adequately appreciated that either temperature changes or ionic strength changes can, in principle, provide a driving force for the occurrence of conformational transitions, for example those illustrated in Fig. 6. Thermodynamics, while not providing any information on the detailed structural organization of molecules, does however, give a body of mathematical correlations between all the properties of the system and is able to identify the "molecular domains" of biomacromolecules, which are relevant for the energetics and the structural organization, let us say, of single or double helical conformations, as for globular proteins (see, for example, Privalov 1980a, b). Analogously, the thermodynamics and the solution behavior of linear ionic polysaccharides in helical conformation seem

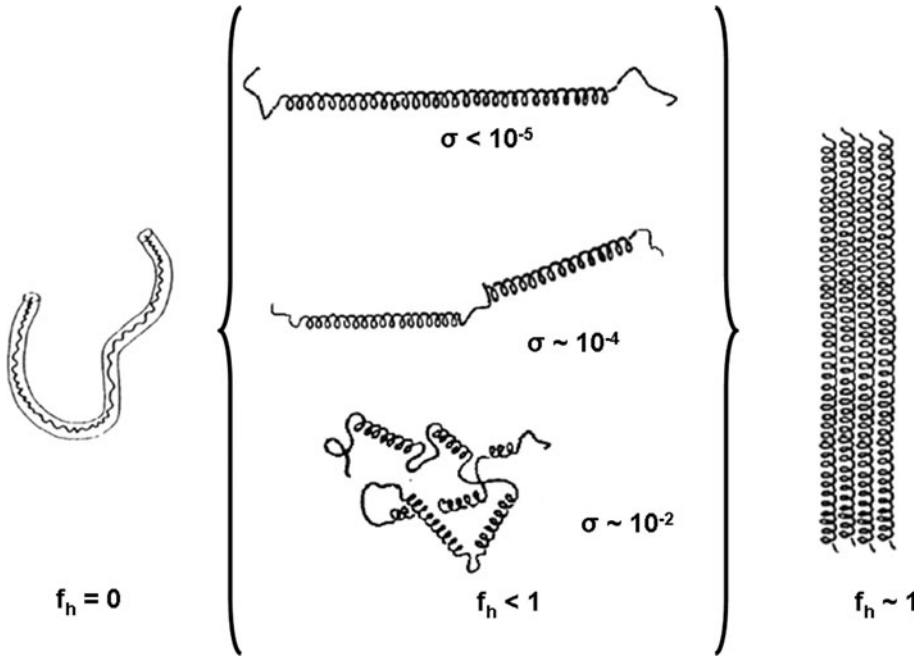

Fig. 6 Temperature or charge density-driven conformational transition in ionic polysaccharides undergoing cooperative coil to helix transformation and successive chain–chain aggregation (examples are treated in Cesàro et al. 2004). The helical fraction f_h, and cooperativity data for several of the models, are also shown

to conform well to the prediction of the statistical theories based on the Ising model (Poland and Scheraga 1970; Cesàro et al. 2004).

Mixed polysaccharide interactions: gelation, phase separation, coacervation, and aggregation

Chain-chain interaction in polysaccharides

Understanding the solution phase behavior and associative phenomena of mixed biopolymers, and polysaccharides in particular, is important from many viewpoints. The thermodynamic phase boundary location and the dynamic aspects of phase separation are related to the non-equilibrium relaxation processes that follow the perturbation of the system from a thermodynamically stable to a thermodynamically unstable state. These transformations are usually induced by changes in temperature, solvent (concentration or composition), or pressure. The phase separation can be monitored by simple turbidimetric measurements or by time- and angle-resolved light scattering, revealing the mechanism of the separation process to be nucleation and growth or spinodal decomposition. Polymer compatibility, in the sense of thermodynamic stability, depends in a subtle way on molecular properties, both intrinsically conformational and energetic. As a general principle two high-molecular-weight polymers are mutually incompatible in the absence of favorable interaction. In other words, a small endothermic interaction between coiled chains segments is sufficient to result in immiscibility of the polymers, in addition to complex topological reasons originating from excluded volume effects. Of great practical interest is the situation in which one or both polymers are weakly charged and modulation of the solution compatibility can be achieved by tuning the ionic strength or pH. Some aspects of these phenomena are reported in the following sections, in particular for weakly charged polysaccharides.

Gelation with phase separation

Many polymers have elements of topological complexity in their ordering processes which make the event of phase separation more complex. Ordering and gelation occur simultaneously in many biopolymers and are usually regarded as intrinsic and intriguing properties worth studying both for scientific and applied reasons (for an extensive review of the fundamentals and of several study cases, see te Nijenhuis 1997). The actual interest here is in the gelation processes occurring in mixed systems undergoing phase separation. In a ternary phase diagram (two different biopolymers and a solvent), several features are relevant to the morphology of the system for each composition and temperature. In the three-dimensional diagram, the most important are the binodal and spinodal surfaces that separate the (stable) biphasic region from the stable monophasic region (Sperling 1997; Koningsveld et al. 2001). When crossing these surfaces, phase separation may not occur immediately and the elapsed time enables the morphology to be tailored by tuning the thermal route. It turns out that the kinetics of isothermal relaxation (curing) and non-isothermal re-arrangements (ordering and/or gelation and phase separation) become determinants of the structure of the final product. However, many physicochemical features are still partially grasped for quantitative understanding of the driving forces and dynamics which ultimately govern all transformations. As far as the morpho-structural characterization of these biopolymer composites is concerned, interaction data, phase transitions, and kinetics of the disorder–order processes clearly point to chain incompatibility at a molecular level (Norton and Frith 2001). A schematic 3D diagram showing equilibrium phase separation and gelation for the maltodextrin–gelatin system under dilute conditions is provided in Fig. 7. The diagram shows the conic section separating the soluble phase from the phase-separated system (cooling line a) and horizontal planes of the transition temperatures identifying the gelation upon cooling of the two biopolymers, at temperatures T_A and T_B, respectively. The kinetics of gelation can be sometimes quite slow if the gelling polysaccharide A is made of maltodextrin, which at temperatures below T_A is maintained in a metastable conformationally disordered phase for many hours. Therefore,

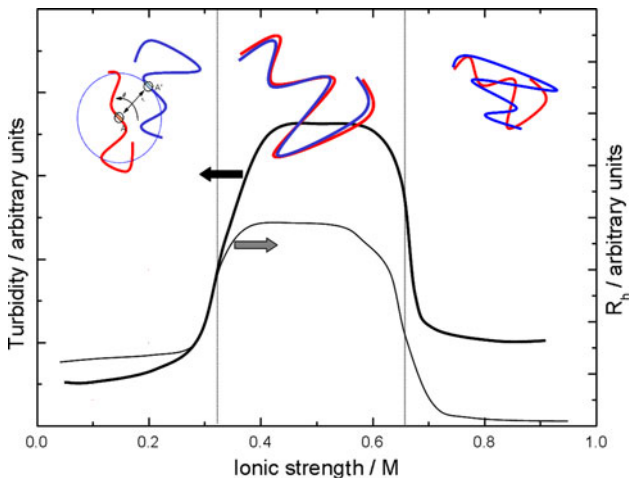

Fig. 7 Portion of a 3D diagram showing equilibrium phase separation and gelation as a function of temperature in dilute solution (100% water at *left side*). The diagram shows the conic section separating the soluble phase (*blue arrow at left*) from the phase-separated system (*red cooling arrow a*) and the horizontal planes of the transition temperatures identifying the gelation upon cooling of biopolymers A and B at temperatures T_A and T_B, respectively

rapid cooling induces phase separation with gelation of biopolymer B (e.g., carrageenan or gelatin) whereas gelation of the maltodextrin occurs after a delay. The complexity of the phase separation is well illustrated by changes in the texture of the system which develop for different thermal histories, as shown by synchrotron-based FTIR spectromicroscopy (De Giacomo et al. 2008).

Coacervation and aggregation

The formation of "soluble" aggregates involving biopolymers has been repeatedly reported in the literature and has prompted several investigations studying the effects of pH, ionic strength, charge density (Wang et al. 1999, 2000), and of the "critical" stoichiometry on coacervate formation in protein–polyelectrolyte and micelle–polyelectrolyte systems (Leisner and Imae 2003; Bohidar 2008 and references therein). Dealing with weak oppositely charged biopolyelectrolytes, the phenomenon called "associative phase separation" is often encountered. More appropriately, such a phenomenon has been envisaged as the overlapping of two processes: one of which is coacervation and the other segregation.

This extensive work has, on the one hand, triggered the development of several interesting applications of the coacervation process (e.g., protein separation and enzyme immobilization) and, on the other, contributed to establishing the phase boundaries governing the liquid–liquid phase separation and the precipitation of oppositely charged polyelectrolytes, including the original phenomenon of "complex coacervation" (Veis 1970). It was noticed that arguments from Flory theory predict that two interacting polymers form complexes, precipitate, or coacervate on the basis of the heterotactic polymer interaction parameter. Since then, several interpretations of the electrostatically driven complexation process have been proposed (Ahmed et al. 1994) and recently reviewed (Cooper et al. 2005) by Dubin and co-workers, recognizing, in particular, the entropic significance of counterion release when associative phase separation occurs.

These considerations are at the basis of the phenomena sketched in Fig. 8, where cases of compatibility and of coacervation are reported. The peculiar feature of "soluble complexes" rests on their ability to undergo structural rearrangements via polyelectrolyte exchange reaction with the polymer chains present in solution. The coacervate solutions have been also described as "soluble complexes" requiring particular conditions, for example the presence of one component with weak ionic groups and a "mismatch" of opposite charge topology. If the latter conditions are prevented or suppressed, the soluble complexes will evolve into the formation of large-scale aggregates (Zintchenko et al. 2003). The situation, however, also can be more

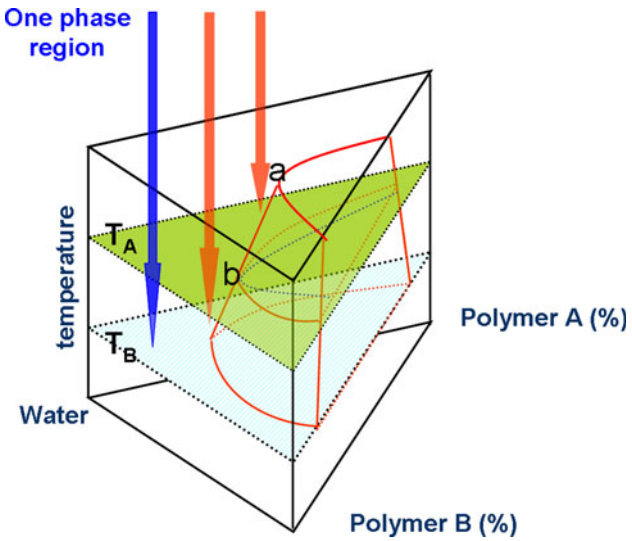

Fig. 8 Phase behavior, as detected by turbidimetry (*left axis*) and average chain dimension, R_h, (*right axis*), as a function of increasing ionic strength for a mixture of two polyelectrolytes of opposite charge and quite different charge density or molecular weight. The molecular sketches represent segmental interactions between the chains, as indicated in the *panel on the right*

complicated when dealing with some biopolymers, as the supposedly random conformations may evolve into helicoidal conformations which are transformed into supramolecular structures or undergo gelation (Cesàro et al. 2004), as illustrated in the another part of this paper.

Turbidimetry is the simplest method for obtaining evidence of the dimensional stability of the system and for detecting well defined transitions between phases as a function of ionic strength. Generally, the phase behavior as a function of increasing ionic strengths (up to approx. 1 M) includes regions of soluble complex formation, coacervation, and soluble mixed system (Fig. 8). Coacervate corresponds to the central phase, whereas the neighboring regions correspond to soluble complexes or fully screened chains. Experimental observations of biopolyelectrolytes (PEs) interacting with micelles support the following statements:

1. coacervates arise from the interaction between micelles and PEs;
2. micelle–PE interaction is electrostatic and therefore controlled by micelle charge, polymer charge, and ionic strength;
3. soluble complexes are precursors of coacervates; and
4. coacervation occurs when soluble complexes have a net charge near zero.

Also of great interest are the molecular weight and temperature dependence of coacervation. Both experiment and theory show this phenomenon is always enhanced by an increase in the PE molecular weight.

Relevance of the correlation between structure and function

In very dilute solution the viscosity behavior of polymers is mainly determined by their conformation (size and shape). Experimental evidence supports the early hypothesis that chain conformation and rigidity can be qualitatively predicted on the basis of viscosity data. Although a wide range of rheological experiments, including small deformation oscillatory, steady, and transient shear, have been performed at finite concentrations and under well defined experimental conditions (Lapasin and Pricl, 1995), little effort has been devoted to attempts to correlate the rheological behavior (slow dynamics) of polysaccharide solutions in terms of chain conformational dynamics.

Generally, the lack of ordered secondary structure leads to entangled networks in which topological constraints govern rheological behavior. Ordered regions along the polysaccharide chains seem to be the prerequisite to establishing intermolecular interactions which lead to non-transient polymer networks. Whenever ordered conformations are stable in solution, both solution properties and rheological behavior have features that arise not only from stiffening of the chains in the ordered conformation but also from the likely interchain interactions between the ordered "array(s)" of chains. The strength of these interactions is reflected in the behavior of the gel (weak or true gel). The effect of polymer conformation and rigidity on the rheological properties is especially evident in the semi-dilute concentration range.

Technologically, it is relevant to mention the importance of polysaccharides in the food industry. Polysaccharide thickeners are widely used to modulate and to control the texture and mouth feel of viscous food systems, ranging from sauces to beverages to dairy products. Several in-mouth-perceived textural characteristics, for example thickness and sliminess of polysaccharide food models, have been successfully correlated with rheological data; the correlation, however, was found to depend on polysaccharide conformation. In fact, disordered random-coil polysaccharides and rigid ordered polymers, for example xanthan, gave different responses. In addition to texture perception polysaccharide conformation and related rheology are of crucial importance to flavor release. It is well known in the food industry that the quantity of flavoring required to produce the same subjective flavor intensity is often much higher in thickened or structured products than in fluid systems. Similar suppression of perceived intensity is also well established for taste attributes (sweet, sour, bitter, and salty). Random coil polysaccharide solutions, ordered polysaccharide weak gels, and true gels have relevant differences in flavor and/or taste release. In particular, the greater viscosity of ordered and/or gelling polysaccharides hinders the mixing process by which flavor and/or taste molecules diffuse, a phenomenon that has a large application in drug delivery also, as presented in the next section.

Carbohydrate-based functional carriers

Biopolymers, and in particular polysaccharides, have several applications in all fields related to human uses. As already mentioned, the oldest application of polysaccharides is obviously in food technology, in which they are naturally involved in formation of the proper texture and have also been used as thickening or gelling agents, for example in juices and chocolate. Nowadays, present and potential advanced applications of polysaccharides in industry are based on exploitation of polyelectrolytes and polyampholytes in mixed systems, for example phase separated nanogels or multilayered membranes, to improve the stability of emulsions. Product microstructure in the cosmetics and pharmaceutical industries is a crucial issue for both the functionality and the quality of the product in terms of final performance (Goddard and Gruber 1999; Kwon and Gong 2006), for example active principle loading and release control. The great advantage of the technological use of mixtures of biopolymers (which can be regarded from the macromolecular point of view as interpenetrating polymer networks, IPN), lies in their intriguing textures, which are very distinct from those created by the individual components (Sperling 1997).

The objective of this final part of the paper is to report on some advanced applications, mainly pharmaceutical and biomedical.

Encapsulation in a hydrogel matrix

In bio-oriented applications, polysaccharides are mainly used as immobilizing and protective structures (capsules, scaffolds,…) for sensitive materials. Encapsulation is a very good example of protection and, among existing preparation techniques, (micro)encapsulation by ionotropic gelation exploits the capability of the polysaccharides to form hydrogels in the presence of appropriate multivalent counterions (for example calcium ions for alginate or triphosphate for chitosan), leading to the formation of a 3D network. In gel particle technology, three methods of preparation of microspheres by ionotropic gelation are commonly described in literature:

1. dropping the polyelectrolyte solution into a solution of small ions;
2. via a w/o emulsification technique; and
3. complexation of oppositely charged polyelectrolytes by mixing and additional coating procedures.

Encapsulation in polysaccharidic hydrogels enables the protection of a wide range of materials of biological interest, from small molecules and protein (enzymes, hormones,…) to cells of bacterial, yeast, and animal origin. The protection is because of the embedding effect of the polymeric matrix, which creates a microenvironment in the capsule able to control the interactions between the internal and the external parts (Smidsrød and Skjåk-Bræk 1990; Thies 2005; Brun-Graeppi et al. 2011). It is also generally accepted that protein-drug release from hydrogel matrices prepared as described above occurs by two main mechanisms, diffusion of the protein through the pores of the polymer network and degradation of the polymer network (Gombotz and Wee 1998). In addition, water diffusion (and swelling, when applicable) through the polysaccharidic hydrogel has also been considered as one of the major factors affecting the rate of drug release (Faroongsarng and Sukonrat 2008).

All bio-based and natural polyelectrolytes, including post-polymerization modified polymers, are the elective candidates for in vivo application (food, pharmaceutical, and biomedical).

Carbohydrate-based materials

Several gelling polyelectrolytes are used commercially, including proteins (e.g., gelatin) and polysaccharides (e.g. chitosan, hyaluronan, alginate, pectin, and gellan) (Aspinall 1983). Chitosan is essentially a polyglucosamine varying in degree of deacetylation, molecular weight, viscosity, etc. The presence of a variable number of amino groups and substitutions may tune the interaction of this polymer with anionic and polyanionic systems, thereby resulting in a wide range of physicochemical characteristics of such complexes. Hyaluronan, a copolymer of glucuronic acid and *N*-acetylglucosamine, is the main component of the extracellular matrix. Alginate is a polysaccharide of algal and bacterial origin. It is composed by units of D-mannuronic acid (M) and L-guluronic acid (G) in the form of homopolymeric block structures along the chains, namely M-blocks and G-blocks, interspaced by alternate MG sequences. Pectin is mainly composed of D-galacturonic acid, which in nature can be methylated, in addition to having small amounts of other neutral sugars interspersed in the chain. Several other polysaccharides from microbial (non-pathogenic) sources have been identified and widely used in pharmaceutics and food uses; examples include gellan and xanthan which have regular structures and efficient properties as viscosity modifiers and gelling agents.

In encapsulation technology, biopolyelectrolytes are often used as combinations of materials, to match gelling properties with other characteristics (for example bioactivity) for the encapsulating system or to further control the release properties. Some examples are the preparation of formulations with controlled release properties based on the association with cellulose derivatives (Lee et al. 2003) and the production of bioreactors for cell (chondrocytes) seeding based on the combination of alginate with hyaluronan and derivatives (Gerard et al. 2005) or chitosan derivatives (Marsich et al. 2008). Moreover, the encapsulation process enables the combination in the capsule of several compounds simultaneously, thus generating a microenvironment which strongly affects the gel-forming process and which can lead to unconventional gelling conditions which affect the formation of the hydrogel matrix (Borgogna et al. 2010).

Despite the wide range of encapsulation techniques, researchers and technologists in the pharmaceutical field are still searching for appropriate drug-delivery systems, and in the era of genomics and proteomics, these challenges are extended to drug molecules of relatively large dimensions (therapeutic polypeptides and proteins), poor lipophilicity, and high susceptibility to inactivation. In this sense the use of nanotechnology principles and nanometric carriers (including nanospheres, nanocapsules, nanomicelles, nanogels, etc.) is a promising solution (Pinto Reis et al. 2006) and has been the objective of many research projects. Nanoparticle drug-delivery systems have several advantages (Liu et al. 2008), for example the ability to penetrate cells and tissues, the possibility of improving the utility of drugs and reducing toxic side effects, while maintaining the ability to control release properties because of the biodegradability of the materials and their sensitivity to conditions such as pH, ion strength, and/or temperature. Rationalizing the assembly mechanisms and tailoring the size, charge, and loading capability to desirable levels are both essential objectives needed to advance biodegradable polysaccharidic nanoparticles as efficient drug-delivery vehicles.

Because nano-organization is relevant to many functional properties, more general comments should be made about the human uses of "nano-particles". Irrespective of the constitutional characteristics, size effect is unavoidable. "Particle mass concentration" has been the most common metric used in all previous drug research. Although it is not always possible to predict effects on the basis of size or surface area alone, there is strong evidence of correlation between biological effects and the dimensional and/or surface properties of nanoparticles. Changing the size by one order of magnitude (e.g., from 100 to 10 nm) results in a change in the number of particles of three orders of magnitude and an increase in the total surface of one order of magnitude. Thus, in many cases, the objective of enhanced interaction with cells can be more profitably achieved by tailoring the appropriate polyelectrolytic surface coating than by reducing nanoparticles to very small size. On the other hand, the reduced dimensions of

nanoparticles can be a source of deleterious effects. Despite the increased application of nanoparticles, generally of synthetic origin, their possible toxic health effects are still not completely understood. In particular, the question of the fate of carriers is often ignored by the chemist tailoring nanostructures. There is a possibility that the very small particles may have unique surface properties in their nanosized form and may be toxic, causing adverse health effects. (Bawarski et al. 2008).

The issue cannot be suppressed, because it involves both worker exposure and societal (ethical) aspects. The easier molecular approach in the construction of suitable nanostructures properly responding to stimuli and releasing drugs has the counterpart of difficulty in transferring laboratory experiments to real patients because of the intrinsic toxicity of non-biological materials. To stress this point, many biopolyelectrolytes are already available and could be used by renouncing to the fine structural chemistry in favor of less symmetrical objects with higher affinity for cells and tissues. Therefore, polysaccharide-based nanoparticles are now an innovative means of drug delivery, with the peculiar property of enhanced bioadhesivity (Goycoolea et al. 2009). From these considerations it is apparent there is a need for extensive biophysical characterization of these complex systems, including investigations with large infrastructures, as outlined in the white book GENNESYS (Cesàro 2009), to correlate physicochemical properties with toxicological effects (Buzea et al. 2007).

Promising systems warranting biophysical investigation

Different types of polysaccharide particle have been developed and described in recent years. These systems have been prepared by exploiting both the formation of ionotropic hydrogels and polyelectrolyte complex (PEC) dispersions, or a combination of the two methods. Because PEC and ionotropic hydrogels are formed by ionic interactions, they have pH and ion-sensitive swelling properties, a high water content, and high electrical charge density (Rajaonarivony et al. 1993; Calvo et al. 1997; Berger et al. 2004). The formation of chitosan hydrogels by ionotropic gelation and polyelectrolyte complexation is an interesting alternative to covalently crosslinked hydrogels. Such systems are biocompatible networks with interesting swelling characteristics. Among the increasing number of successful polysaccharidic formulations developed so far, it is worth mentioning, as representative examples, the preparation of insulin-loaded chitosan/alginate nanoparticles for insulin nasal delivery in diabetes therapy (Goycoolea et al. 2009), the synthesis of antigen-loaded chitosan nanoparticles for hepatitis B vaccination (Prego et al. 2010), and the preparation of chitosan–hyaluronic acid nano-carriers for efficient gene delivery (Duceppe and Tabrizian 2009).

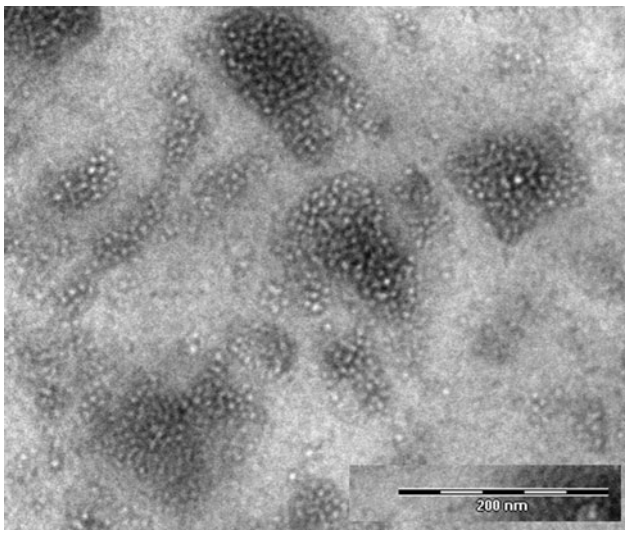

Fig. 9 Transmission electron microscope (TEM) micrograph of nanoparticles of less that 25 nm obtained by ionotropic gelation of a low-molecular weight un-derivatized chitosan with addition of TPP under slightly acidic conditions

Figure 9 shows the typical shape of nanoparticles obtained by ionotropic gelation of a low-molecular-weight un-derivatized chitosan with addition of triphosphate ions (TPP) under slightly acidic conditions (Rampino 2011). These nanoparticles can have an initial size as low as approximately 25 nm, and undergo Ostwald ripening in the absence of suitable co-solutes to reach a final stabilized size of approximately 250 nm. One of the main advantages of this system is preparation under mild reaction conditions; the main drawback is difficulty of production on a large-scale. Several factors affect the formation of chitosan nanoparticles, and must be carefully controlled: solution pH, temperature, ionic strength, order of mixing, flexibility of polymers, molecular weight, and degree of deacetylation of chitosan (Berger et al. 2004).

Thus, a large community of scientists and technologists now has the know-how to produce effective nanoparticles with sophisticated complex structures that, however, need intensive physical investigation, as has been done for elementary structural units over the last 40 years.

Conclusions

Topological and dynamic aspects of the average conformational properties of polysaccharides have been described, with the intention of assessing the utility of these biopolymers in the construction of nanostructures with applications in food, cosmetics, and pharmaceuticals. The main reasons for this attention to polysaccharides are certainly their versatility and complete biocompatibility and

biodegradability. It has been also stressed that additional, and often not yet explored, performance may be easily achieved by use of a long list of potential derivatives, in which pendant moieties are introduced to modify the solution thermodynamics and conformation.

The presentation has been organized semi-tutorially, from the unitary chemical blocks to chain topology and to supramolecular structures, providing some hints of how the final properties of the products may be altered. The variety of phenomena and models dictates the organization of the paper; still, among many others, the following issues can easily be raised:

- How do water dynamics control chain–chain interaction and modify local conformation and dynamics?
- How do local dynamics affect the long-range order and stability of supramolecular structures?
- How is diffusion of small molecules, including peptides, flavoring molecules, or nutraceuticals modified by changing the nature of the polysaccharide chains?

The paper does not provide any answers to these questions, but it provides the background for topical understanding of the properties of the polysaccharide chain.

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