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Biopolymer Surfactant Interactions

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1. Introduction

Biopolymers are polymers that are biodegradable. The input materials for the production of these polymers may be either renewable (based on agricultural plant or animal products) or synthetic. There are four main types of biopolymer based respectively on: **1. Starch 2. Sugar 3.Cellulose and 4.Synthetic materials.** Two main strategies may be followed in synthesizing a polymer. One is to build up the polymer structure from a monomer by a process of chemical polymerization. The alternative is to take a naturally occurring polymer and chemically modify it to give it the desired properties. A disadvantage of chemical modification is however that the biodegradability of the polymer may be adversely affected. Therefore it is often necessary to seek a compromise between the desired material properties and biodegradability. Current and future developments in biodegradable polymers and renewable input materials focus relate mainly to the scaling-up of production and improvement of product properties. Larger scale production will increase availability and reduce prices.

Surfactants are wetting agents that lower the surface tension of a liquid, allowing easier spreading, and lower the interfacial tension between two liquids. Surfactant solutions have a general tendency to solubilize a certain amount of additives, which can be correlated with their structural organization and mutual interactions.

Polymer-surfactant interactions [1-8] have been extensively investigated by researchers due to its manifold applications in the fields of the food industry, pharmaceutical industry and analytical biochemistry. The interaction of protein with other ingredients, mostly cationic and anionic surfactants, is of particular interest because they are used co-operatively in formulated complexes. The mechanisms for the protein-surfactant interactions are polyelectrolyte absorption [9], hydrophobic [10] and ionic interactions [11], depending on the substrate and type of proteins involved. The effect of adding a hydrophilic counterion, NaCl to aqueous CTAT solution was measured by dynamic light scattering. The obtained results indicate that the micelles grow rapidly upon salt addition and eventually achieve a constant size under static conditions [12]. The results were explained in terms of a competition between micellar growth induced by salt addition and changes in micellar flexibility caused by ionic screening effects. Adding hydrophobic non-polar additives to cationic surfactant solutions is also found to increase the micellar growth [13, 14].

Several research papers on the micellar solution of cationic surfactant and salt systems are available [15-31]. Many cationic micellar solution were prepared from mixing long-chain

cationic surfactants such as hexadecyltrimethylammonium (CTA$^+$) and hexadecylpyridinium (CPy$^+$) with organic counterions such as, salicylate, Sal$^-$,tosylate T$^-$)), and (HNC$^-$) [32-40]. For the above systems, the growth of the cationic micelle was mainly due to the strong binding of hydrophobic counterions. As the hydrophobic character of the cationic surfactant increases the cmc, degree of counterion dissociation (α) and Gibbs free energy of micellization, ΔG_m, decreases while the aggregation number, N_{agg} increases. The cmc, α, ΔG_m and N_{agg} values of cationic surfactants CTAC, CTAB and cetyltrimethylammonium tosylate, CTAT at 30°C were compared [41]. It is noted that cmc, α and ΔG_m were lowest while N_{agg} is highest to CTAC and CTAB. This correlation between cmc, α, ΔG_m, N_{ag} and type of counterion indicate that the micellization process is more spontaneous in the case of CTAT compared to CTAC and CTAB. From an application point of view, the higher binding of T$^-$ to CTA$^+$ explains the higher adsorption of CTAT onto silica [42]. It was concluded that on the silica surface, increasing the binding degree increases the cooperativity of adsorption below the bulk cmc, corresponding to a stabilization of interfacial self assembly. An increase in the surface excess concentration due to a decrease in the curvature of the adsorbed aggregates is noticed. The effect of adding a hydrophilic counterion NaCl to CTAT aqueous solution was measured by dynamic light scattering. The obtained results indicate that the micelles grow rapidly upon salt addition but eventually achieve a constant size under static conditions [43]. The results are explained in terms of a competition between micellar growth induced by salt addition and changes in micellar flexibility caused by ionic screening effects. Adding hydrophobic non polar additives to cationic surfactant solutions is also found to increase the micellar growth [44, 45].

There are many evidences for an entangled micellar phase in several aqueous cationic detergent systems in the presence of added salt [46-49]. The presences of neutral salts [50-53] showed a marked shift in phase transitions. It has been suggested that the protein unfolds in the cooperative binding region [54] and sodium chloride is reported to act as a denaturing agent in many protein systems [55-57]. According to Curtis et al. [58] in classical salting-out behavior, the protein-salt preferential interaction is unfavorable as addition of salt raises the surface free energy of the protein, thereby protein-protein attraction increases leading to a reduction in solubility. Systematic investigations of solubilisation of gelatin in AOT-isooctane-water microemulsions in high concentrations was found to be analogous to solubilization of an ionic polymer in a surfactant solution by Koteshwar et al. [59] where surfactant polymer interactions play a dominant role.

There would be definite interactions between the negatively charged surfactant and the biopolymer, since gelatin is amphoteric due to the presence of both carboxylic and amino groups. The organogel thus formed could well be described as a surfactant-polymer complex [60-62]. It is noted that, on increasing the surfactant concentration, this "complex" would redissolve in excess surfactant solution, and the rigidity of the system would be affected. This could lead to the formation of a gel with a flexible network and the interesting physical properties could be exploited for biotechnological applications. The viscous organogels formed from the solutions containing AOT, isooctane, water, and high amounts of gelatin (up to 6.8%) at high temperature (>40°C) were reported [63] to actually "melt" on cooling, and at low temperature (<10 °C), they are free-flowing liquids of moderate viscosity. On rewarming, these solutions progressively become viscous with increasing temperature. The phenomenon is completely reversible, and again on cooling the viscous gels becomes free-flowing liquids. The phenomenon is observed not only in the case of a surfactant with a negatively charged headgroup such as AOT but also in a surfactant such

as CTAB with a positively charged headgroup. Such behavior is uncommon and very interesting from both the biophysical and biotechnological points of view. In solutions of nonionic surfactants, such as Tween 80-Tween 60 mixtures, solubilization of gelatin was too low to form an organogel.

Cationic CTAB forms worm-like micelles with Cl- and Br- ions due to counterion binding and charge screening but rod-like micelles with salicylate- ions are formed as a result of charge attraction, counterion binding, and hydrophobic interactions [64]. In dilute solutions, the length of rod-like CTAB: NaSal micelles can exceed 1000 A° [65] while that of worm-like CTAB: KBr micelles are around 500 A° [66]. These rod-like surfactant micelles display interesting rheological properties, which result from their polymer-like structure. In many applications, such as shampoos [67] and drag-reducing agents [68], rod-like micelles are used as viscosity modifiers. Over the same period of time, extensive studies have been conducted on interactions between water-soluble polymers and surfactants.

Schubert et al. [69] examined the microstructure and rheological properties of mixed CTAT and SDBS micelles. The effects of surfactant ratio on the addition of the sodium chloride and sodium tosylate were noted. Small angle neutron scattering studies revealed that the addition of sodium chloride induces electrostatic screening between micelles and reduces or eliminates intramicellar repulsions. Sodium tosylate had a similar effect at low concentrations; however, further addition resulted in the formation of branched micelles. The formation of branch points was the result of interactions between the surfactant and the added hydrotrope, and was not observed with the addition of sodium chloride. Varade et. al.[70] reported that the addition of three anionic hydrotropes affected the cmc, viscosity and CP temperature of the cationic CTAB, the anionic SDS and the nonionic polyoxyethylene t-octylphenol (Triton X-102). All the hydrotropes increased the CP of Triton X-102, while the sodium chloride decreased the CP. The cmc of CTAB decreased and the viscosity of concentrated surfactant solutions rose sharply with the addition of any of the hydrotropes. The decrease in cmc suggests an increase in micelle stability and a strong increase in solution viscosity is indicative of micelle elongation.

However, until now, stabilizing effect of salts in a gelatin-micellar media has not been reported. Physico chemical or biological treatments [71, 72] trigger protein modification i.e., modification in conformation/structure and there by functional properties of protein, leading to partial hydrolysis or aggregation of gelatin. The gelatin conformation is retained in many formulations to improve the functional properties in combination. The present work explores the effect of NaCl on the conformational properties and stability of gels formed by polyelectrolyte-surfactant–water interfaces stabilized by gelatin–surfactant complexes. This study is attempted to unravel the effect of brine on the gelatin-surfactant (CTAB) system by viscosity, conductivity, Circular Dichroism (CD) spectroscopy, Differential scanning calorimetry (DSC) and by Fourier Transform Infrared Spectroscopic (FTIR) measurements at 35˚C.

2. Experimental procedure

i.   **Materials and Methods:** An alkali processed deionized bone gelatin (Loba Chemie, India), Cetyl trimethyl ammonium bromide (CTAB) and sodium chloride (NaCl) were used as supplied.

ii. **Sample Preparation:** Gelatin solutions were prepared by weighing the required amount of gelatin flakes and soaking in hot water (~40°C) with stirring. Deionized distilled

water from Millipore (Milli-Q; 18 MΩ) was used for all the experiments. The concentrations quoted here are in weight percentage of gelatin. The surfactant solution was prepared by weighing out the requisite amount each time and dissolving it in a freshly prepared gelatin solution. The sample solutions after proper mixing were left for equilibration for 24 hrs.

Viscosity and CD measurements were done for the pure aqueous solution of gelatin and for the samples were done at 35± 2°C. Flow characteristic were carried out by Capillary Viscosity method and were measured using an Ostwald viscometer thermostated at 35 ± 2°C. Under Newtonian condition density correction is not made since these are found negligible. The solutions were kept at the experimental temperature for at least 30 min to attain thermal equilibrium. At the ambient temperature, gelatin is characterized macroscopically as a random coil in dilute solutions with a length of ~20 Å.

3. Influence of salt on cationic surfactant – biopolymer interactions in queous media

Changes in the bulk viscosity are caused by structural changes in the gelatin/surfactant associates, for example, by formation of intermolecular cross-bonds. Figure 1 shows the variation of relative viscosity, η_r of CTAB in presence of sodium chloride in 1.5% gelatin. A sharp increase in relative viscosity as a function of [CTAB + NaCl], expressed in terms of mole fraction of the salt, is observed. The abrupt increase in viscosity can be the onset of an association of gelatin with CTAB. The micelles at this stage act as transient cross-links i.e., inter polymer association are possible. According to Mukerjee [73], an additive, which is surface active, will mainly be solubulised near the micellar head group region, and facilitate the sphere→ higher order transition with a concomitant increase in η_r and the packing parameter, Rp.
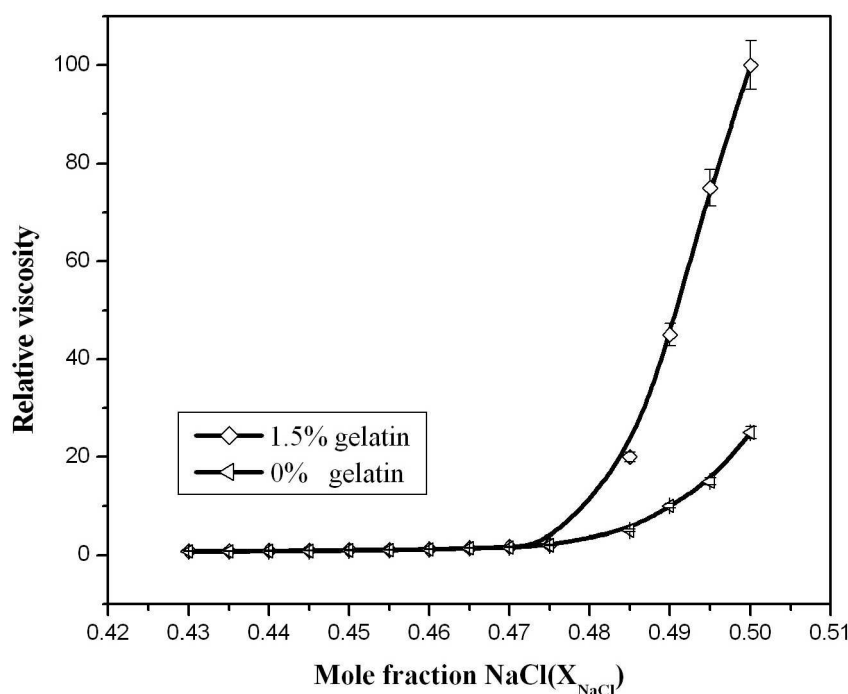

Fig. 1. Variation of relative viscosity, ηr, of [CTAB + NaCl] in 1.5% gelatins at 35 ◦C.

Gelatin is a polypeptide with 12% negatively charged amino acid residue is a random coil in dilute solutions with a persistence length of ~ 20A^0 and R_h ~ 220 A^0. DLS studies of CTAB – Gelatin interaction [74, 75] showed that beyond critical aggregation concentration gelatin – CTAB complexes were observed to grow significantly and were in equilibrium with CTAB micelles. The micellar shapes were found to be oblate ellipsoidal for CTAB micelles which were explained through the necklace –bead model through co-operative bonding [76]. Hydrophobic interaction may be operating between the hydrophobic part of CTAB and hydrophobic sites on the gelatin chain. Beyond CMC, the co-operative binding prevailed and gelatin – CTAB complexes were found to co-exist with free CTAB micelles. Increase in size of the hydrodynamic radius of Gelatin-CTAB complex is due to electrostatic repulsion between the positive sites of the chain and bulky cationic head groups. Hydrophobic interaction may be operating between the tail part of CTAB and the hydrophobic sites on the Gelatin chain. An abrupt increase in, ηr, can be due to the incorporation of halide ion leading to the screening of the electrostatic interaction of surfactant head groups. Decreased head group repulsion favors closer packing of the surfactant monomers and hence induces sphere → rod transition in micelles [76, 77]. At higher [CTAB+ NaCl], further increase in size is observed due to formation of polymer –micelle complex. Further, the interfacial portioning of additive is important for viscosity rise (micellar growth) while core solubilization (swollen micelle) enhances the sphericity of the micelle and works oppositely. The high viscosities observed in these systems are interpreted in terms of a micelle sphere-to-rod transition, which occurs over a certain range of mconcentration of either surfactant or added salt.

To gain information on the secondary structure present in gelatin in the system, CD measurements were performed at various concentrations of CTAB in the presence of NaCl (Fig. 2).
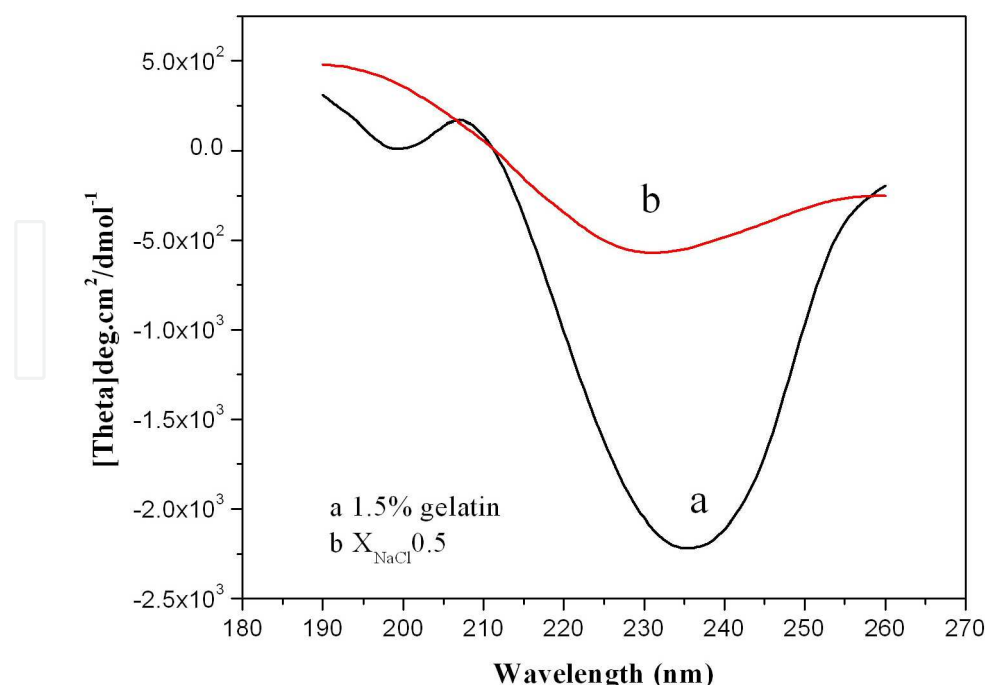

Fig. 2. Circular dichroism spectra on the effect of (CTAB+ NaCl) on gelatin in the wavelength region260 – 190 nm. (a) Gelatin alone and (b) gelatin+0.5M CTAB+0.5M NaCl.

The random coil structure found in gelatin gives rise to a characteristic negative peak at around 235 nm in the CD spectrum, which decreases in ellipticity upon addition of [CTAB +NaCl]. The general shape and peaks of the spectrum does not show much change in surfactant /gelatin/salt mixture of varying concentrations except for their magnitude. Ellipticity was maximum for the highest concentration and negative peaks with lower intensity were observed in comparison to gelatin alone. This implies that the gelatin has no conformational change at 35°C. The lower ellipticity indicates [78] the alteration or disturbance in the order or periodicity in the structure of surfactant i.e., aggregation or disruption as predicted from other measurements. The increase in molar ellipticity at 235 nm for the system is indicative of aggregated structures of surfactant/gelatin in the presence of salt and the effect is very much pronounced at higher concentrations of salt. This shows that the gelatin facilitates the structural transitions in CTAB solution in presence of salt without having any structural changes to it.

The utility of FTIR spectroscopy in studies of micellar growth induced by electrolyte has been increasingly demonstrated in recent years [79, 80]. In order to verify the expected trend of CTAB to alter the ordered structure of gelatin into a random structure especially at low concentrations Fourier transform infrared spectroscopy (FTIR) spectra was carried out for [CTAB + NaCl] in1.5٪ gelatin, which depict certain characteristic features (Fig.3).
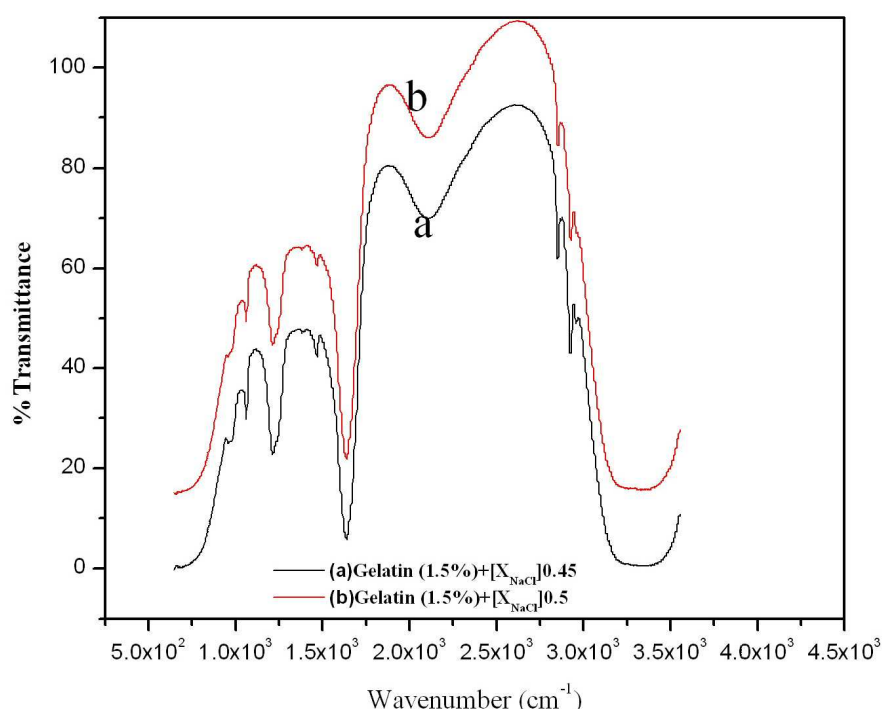

Fig. 3. FTIR spectra of 0.1M and 0.5 M [CTAB + NaCl] in 1.5% gelatin at 35 °C.

The transmittance bands at 3368, 2923 and 2853 cm^{-1} represents the –OH, –CH_2 and –CH_3 aliphatic groups whereas bands at 1466 and 1635 cm^{-1} represent the NH-group bending vibration and vibrations of –OH group in the primary alcoholic group, respectively. The amino group has a characteristic absorption band in the region of 3400–3500 cm^{-1}, which is masked by the broad absorption band from the –OH group. The shoulders at 1635 cm^{-1} represent the C=O groups and suggests gelatin as a partially deacetylated product. The conformation of protein in the interface changes slowly to allow the maximum number of

hydrophobic segments to contact the surface. Above the saturated concentration, the further rearrangement may allow more binding of CTAB to hydrophobic patches on the protein surface. In the process to become hydrophilic, the electrostatic interactions of anionic amino-acid residue to cationic surfactant might have taken place. The sphere-to-rod shaped transition is accompanied by partial ordering of methylene chain.

DSC traces were recorded in a high sensitivity calorimeter, in the temperature range -80 to 80° C, with a heating rate of 5° C / minute. Fig. 4 shows the DSC heating curve for [CTAB+NaCl] in presence of gelatin. The two main transitions associated to the main peak can be explained in terms of different structures in different populations, and the different surfactant packing of CTAB, which determines the vesicle curvature. This study once again strengthens our observation that higher order structural transitions leading to gel formation has happened without undergoing any conformation changes to gelatin.
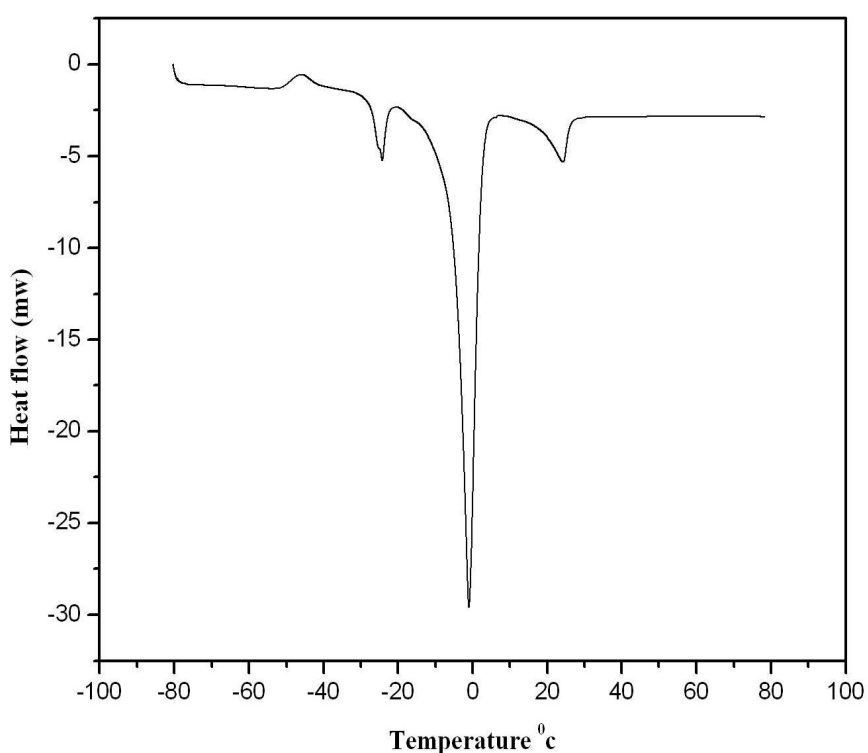
Fig. 4. DSC curve for X_{salt} =0.1M in the gelatin/CTAB/NaCl system.

The large endothermic peak, situated around 0^0c is due to the melting of ice, together with another downward peak at a high temperature (about 25^0c) which reflects the evaporation of the non-crystallisable water which might be an indication of the possible chemical cross-linking [81, 82].

4. Conclusion

The effect of sodium chloride on micellar property of CTAB in biopolymer gelatin were systematically studied. It was found that, micellisation and transition is favoured by increase in concentration of sodium chloride, however, without affecting the conformation of gelatin. The main findings from the present investigation refer to the stabilizing role of salt in presence of a biopolymer, gelatin, in micellar media. Increase in viscosity and gel

formed is further explored by other techniques, such as CD spectroscopy, DSC and FTIR measurements that suggest a sphere - to - higher order micellar transition in the system. These results emphasize new possibilities offered by such systems in obtaining organized assemblies with novel architectures, for investigating the fundamental functional attributes in colloid studies and pharmaceutical studies. Hence, we strongly feel that this work will contribute to extend the field of utilization of gelatin, showing its true potential for specific utilizations in pharmaceuticals.

5. Acknowledgements

6. References

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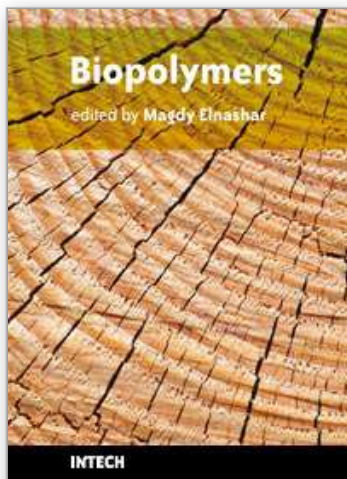
Biopolymers

Edited by Magdy Elnashar

Biopolymers are polymers produced by living organisms. Cellulose, starch, chitin, proteins, peptides, DNA and RNA are all examples of biopolymers. This book comprehensively reviews and compiles information on biopolymers in 30 chapters. The book covers occurrence, synthesis, isolation and production, properties and applications, modification, and the relevant analysis methods to reveal the structures and properties of some biopolymers. This book will hopefully be of help to many scientists, physicians, pharmacists, engineers and other experts in a variety of disciplines, both academic and industrial. It may not only support research and development, but be suitable for teaching as well.

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