FTIR Spectroscopy of Natural Bio-Polymers Blends

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ABSTRACT

Blending natural polymers are considered as important approach towards the production of new biopolymers with unique properties. Blending could enhance surface reactivity and produce unique hydrogen bonding which could produce a new composite with new physical as well as chemical properties. Spectral recognition of such materials could be achieved with the help of molecular modeling techniques such as Fourier transform infrared spectroscopy FTIR. Accordingly this work is conducted to study the molecular structure of chitosan, starch, agarose, gelatin blends with different ratios. FTIR spectra vifiry the formation of blend which could be dedicated for many biological applications.

Key words: Polysaccharides, Natural protein, Biopolymer, FTIR and Molecular structure.

Introduction

Chitosan blending is considered as one of the most attractive and effective biomaterial. The properties of Chitosan/biopolymer(s) blend can be controlled by the characteristics of the introduced side chains, including the molecular structure, length, and number. Chitosan was blended with several polymers such as PVA (polyvinyl alcohol), gelatin, silk fibroin, starch and cellulose (Isogai, 1992; Ratto, 1996; Arvanitoyannis, 1998; Lee, 1999, Park, 1999 Zhang, 2002 and Mangala, 2003). Wound dressing based on aligned material is well known, in literature as well as from commercial point of view, in wound management (Piaquadrio, 1992 and Paul, 2004a&b). Cellulose is a linear polymer that has the same B-(1-4)-D-glucopyranose units backbone as Chitosan, expect for the acetamide is replaced by 2-hydroxy group. It forms crystals where intra-molecular and intra-strand hydrogen bonds hold the network flat allowing for more hydrophobic ribbon faces to stack (Isogai, 1992). This tendency to form crystals utilizing extensive intra- and intermolecular hydrogen bonding makes it insoluble in normal aqueous solutions. Blending cellulose with Chitosan is suggested to be a useful method to improve the mechanical properties of Chitosan (Wu, 2004). Several studies reported that there are in the presence of specific interactions between cellulose and Chitosan molecules based on the analysis of Raman and $^{13}$C NMR spectroscopy,Moreover the X-ray diffraactometry (Hasegawa, 1992a&b; Hasegawa, 1994 and Wu, 2004). (Isogai, 1992) prepared cellulose/Chitosan blend films using trifluoroacetic acid as cosolvent for the two polysaccharides. They reported homogenous mixing between Chitosan and cellulose molecules. (Wu, 2004) prepared blend membranes from Chitosan and cellulose to study blend membranes morphology, mechanical property, transpiration and the antimicrobial capability. The results indicated that the mechanical and dynamic mechanical thermal properties of the cellulose/Chitosan blends are almost dominated by cellulose, indicating that cellulose/Chitosan blends are considerably immiscible. They suggested that the intermolecular hydrogen bonding of cellulose is break down to form cellulose–Chitosan hydrogen bonding; however, the intra-molecular and intra-strand hydrogen bonds hold the network flat. Collagen is one of the most important biopolymers in animals, the abundance and structural properties of collagen has led to a wide range of uses, including healing, sutures and haemostatic ( Miyata, 1992). Collagen possesses characteristics as a biomaterial distinct from those of synthetic polymers; collagen is capable of acting as the basis for biomaterials that can be used to interact with living tissue as it possesses commensurate biomechanical and biochemical properties to living tissue (Sionkowska, 2004a, b). This suggested that the specific properties of each of collagen and Chitosan may be used to produce synthetic blends that confer unique structural and mechanical properties. (Hirano, 2000) prepared Chitosan/collagen fibers that were chemically modified and evaluated for their blood compatibility. The Chitosan fiber has improved when collagen was added to Chitosan as well as when the resultant Chitosan-collagen fibers were N-derivatized. Anti-thrombogenic activity was obtained for collagen coated on N-acyl derivatized fibers. They suggested that Chitosan fibers offer the potential for applications where hemocompatibility is sought. In summary, Chitosan-collagen-GAG was found to be beneficial for skin substitutes (Katalinich, 2001). Gelatin has excellent placticity, adhesiveness, biocompatibility and nonantigenicity (Muzzarelli, 1993 and Cheng, 2003). Gelatin has been shown to exhibit activation of macrophages and high hemostatic effects (Rose, 1989). It has the potential to mix with Chitosan at the suitable pH value due to its ability to form hydrogen bonding with Chitosan. Thus, gelatin was postulated as a suitable candidate blended with Chitosan for biomedical applications. (Mao, 2003) prepared scaffold composed of...
Chitosan and gelatin and studied some physicochemical properties of the produced scaffold to confirm its applicability as an ideal skin substitute. He found that the Chitosan-gelatin scaffolds are more wettable and adsorbed more water than Chitosan alone. The highest water uptake of the scaffold is approximately 35 times that of dry sponges when the ratio of Chitosan: Gelatin is 3:7. (Xu, 2004) prepared Chitosan/starch films by combining Chitosan solution and two thermally gelatinized cornstarches.

The present work is conducted in order to study some important biopolymer blends with the help of FTIR spectroscopy.

**Materials and Methods**

**Reagents**

Cellulose (Cellulose powder, ca. 20 micron), from Sigma-Aldrich, Steinheim, Germany.

Soluble starch, extrapure AR, from Sissco Research Laboratories PVT. Ltd, Bombay, India.

Soluble collagen from Sigma Chemical Company, (St. Louis, MO, U.S.A.).

Agarose L. from Prolabo, by Neosystem Laboratories (Strasbourg, France).

Gelatin pure from Panreac, Barcelona, Spain.

Acetic acid pure for analyses, 96 % ADWIC, Egypt.

Tetrafluoroacetic acid from Merck (Darmstadt, Germany).

Chitosan (s) high molecular weight, from Fluka Biochemika, Switzerland.

Chitosan (p), prepared in the lab., from chitin which is produced from shrimp shells by treating with an aqueous 3-5 percent NaOH solution. The resulting product is neutralized and calcium is removed by treatment with an aqueous 3-5 percent HCl solution at room temperature to get a white or slightly pink participation of chitin. The N-Decacetylation of chitin is prepared by treatment with an aqueous 40-45 percent NaOH solution, and the precipitate is washed with water. Crude sample is dissolved in aqueous 2% acetic acid, and the insoluble material is removed. The resulting clear supernatant solution is neutralized with an aqueous NaOH solution to afford a purified sample of chitosan as a white precipitate (Hirano, 1996).

**Preparation of Blend Films**

Chitosan was mixed with different polymers namely, Starch, Agarose, Collagen, Gelatin and Cellulose to get several blends in the form of films. The mixing ratios are indicated in table 1.

<table>
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<tr>
<th>Chitosan, mg</th>
<th>Polymer Content, mg</th>
<th>Polymer Ratio %</th>
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**Polymer Film Preparations**

The prepared mixtures shown in table 1 were subjected to the following preparation procedures.

1- The mixtures containing chitosan starch, chitosan gelatin, chitosan collagen and chitosan agarose were added to 100 ml acetic acid (7 % solution) at room temperature with stirring to get a homogeneous solution.
2- The solution was distributed into levelled hydrophobic polystyrene Petri dishes (10 cm in diameter). Solution was left to dry for about 48 h at room temperature in the open air, to get the desired films. Complete drying was avoided as some moisture is required for films to remain flexible and not to crack.

3- The films were finally removed (by peeling) from the trays and placed in sealed containers to avoid moisture exchange.

4- Chitosan cellulose mixtures were added to tetrafloroacetic acid and left 48 hours with stirring to get homogenous solutions.

5- Step 2 and 3 were repeated for chitosan cellulose solutions.

6- All the previous steps were repeated for chitosan prepared from shrimp.

**Experimental Techniques**

**Fourier Transforms Infra-Red Spectroscopy (FTIR)**

FTIR is the main technique, which is used in this work. FTIR Spectra were obtained on a JASCO, FTIR- 300 E., Spectrophotometer in the spectral range 400-4000 cm⁻¹, with a resolution of 4 cm⁻¹. Samples were prepared in the form of films.

**Results and Discussions**

When two or more substances are mixed, physical blends versus chemical interactions are reflected by changes in characteristic bands (Yin et al., 1999). Accordingly; blending two polymers is an approach to develop new biomaterials exhibiting combinations of properties that could not be obtained by individual polymers (Ratajska 1998). The analysis of FTIR spectra of each blend enables studying the interactions which possibly takes place.

**Chitosan/Gelatin:** The IR spectra of the Chitosan/Gelatin composite films are shown in figures (1) and (2). The IR spectrum of Chitosan film displayed peaks around 901 and 1155 cm⁻¹, assigned to the saccharine structure and an amino characteristic peak at 1591 cm⁻¹. There was a stronger absorption band at 1651 cm⁻¹ corresponding to the amide of Chitosan. Gelatin film was characterized by its amino band at 1537 cm⁻¹ and carbonyl peak at 1655 cm⁻¹. Incorporation of gelatin led to small shifts in the positions of amide I and amide II of Chitosan toward the higher frequencies.

![Fig. 1. FTIR absorption spectra for, prepared Chitosan, Gelatin, as well as their blends.](image)

This result implies that hydrogen bonding occurs between Chitosan and gelatin molecules in the polyelectrolyte complex formation, which is consistent with the reported results (Jin Shu Mao, 2003 and Mingyu, 2003).
Chitosan/Agarose: FTIR spectra of Chitosan, Agarose and Chitosan/Agarose blends are indicated in figures (3), (4).

![FTIR spectra of Chitosan, Gelatin, Agarose, and blends](image1)

**Fig. 2.** FTIR absorption spectra for, standard Chitosan, Gelatin, as well as their blends.

![FTIR spectra of Chitosan, Agarose, and blends](image2)

**Fig. 3.** FTIR absorption spectra for, standard Chitosan, Agarose, as well as their blends.

As indicated previously Chitosan is an amino glucose characterized by a small proportion of amide groups. This in turn has exhibited a broad band at 3431 cm\(^{-1}\), which is assigned to the N-H and hydrogen bonded O-H stretch vibrational frequencies. Further, in the C-H stretch region of FTIR spectrum, the higher intensity peak at 2923 cm\(^{-1}\) is assigned to the asymmetric mode and the lower intensity peak at 2857 cm\(^{-1}\) is assigned to the symmetric modes of CH\(_2\). In addition, the characteristic band due to CH\(_3\) scissoring, which usually occurs at 1465 cm\(^{-1}\) was also present in the sample. Since the grade of Chitosan used in the present study was calculated as 85\% for both standard and prepared deacetylated, an amide bond peak was present in the spectra and the
C=O stretch of amide bond was observed at 1661 cm$^{-1}$. The peaks at 1550 and 1599 cm$^{-1}$ were assigned to strong N-H bending vibrations of amide II, which usually occur as strong band in the range of 1640 to 1550 cm$^{-1}$. The peak at 1651 cm$^{-1}$, representative of C=O stretch of amide I bond in Chitosan film, is shifted to higher frequencies as a result of blending with Agarose. The same takes place for the N-H bending vibration.

Chitosan/Collagen: Both Chitosan and Collagen are now well known for their interesting biological properties. This observation brings us to the important question of how Chitosan interacts with collagen. Indeed, the answers to this question could be gotten from FTIR spectra of their blends.

![Fig. 4. FTIR absorption spectra for, prepared Chitosan, Agarose, as well as their blends.](image)

The overall conclusion is that Chitosan/ Collagen blends are miscible and interact at the molecular level; new hydrogen bonding networks appear to alter the Collagen helical character and accordingly the overall physical parameters of the blend. In the miscible blends of Chitosan / Collagen, the changes in FTIR spectra are indicated in figures (5) and (6). The difference is clearly indicated as in the modes of vibrations assigned to amide groups. The peaks corresponding to the amide I display a change as the level of collagen is reduced.
relative to the level of Chitosan in the sample. As the level of collagen in the sample decreases, the amide I peak decreases too, until it is present only as a small shoulder to the amide II peak. The amide II peak remains relatively consistent with varying levels of Chitosan/Collagen. The band at 1283 cm⁻¹ persists until the relative amounts of Chitosan/Collagen are equivalent in the sample. The bands at 1204 and 1240 cm⁻¹ persist in the samples until 30% of the sample material is Collagen. Increasing the level of Chitosan in the sample beyond 70% brings about a loss of these two bands.

**Chitosan/ Starch:** Figures (7) and (8) reflect the typical spectrum of Chitosan/Starch composite film. As mentioned; the FTIR spectra of the starch consists of three characteristic peaks between 923 and 1162 cm⁻¹, are attributed to the C=O bond stretching (Goheen 91). The bands at 1659 and 1467 cm⁻¹ are assigned to the (O-H) bendings of water and CH2, respectively (Mano 2003). The sharp band at 2926 cm⁻¹ is characteristic of C-H stretches associated with the ring methane hydrogen atoms.

![FTIR absorption spectra for standard Chitosan, Collagen, as well as their blends.](image1)

**Fig. 6.** FTIR absorption spectra for, standard Chitosan, Collagen, as well as their blends.

![FTIR absorption spectra for prepared Chitosan, Starch, as well as their blends.](image2)

**Fig. 7.** FTIR absorption spectra for, prepared Chitosan, Starch, as well as their blends.

An extremely broad band occurs at 3400 cm⁻¹ due to the hydrogen-bonded hydroxyl groups that contribute to the complex vibrational stretches associated with free inter- and intramolecular bound hydroxyl group, which constitute the gross structure of starch (Fang 2002). In Chitosan the peaks at 2839 and 2925 cm⁻¹ are typical C-H stretch vibrations (Wang 2004). The peak at 1739 cm⁻¹ suggested the presence of carbonyl group, the one at 1633 cm⁻¹ was due to the C=O stretching (amide I), and the peak at 1314 was due to amide III
peaks. The sharp peaks at 1374 and 1414 cm$^{-1}$ correspond to the CH$_3$ symmetrical deformation mode (Sannan 1978). The broad band at 1076 cm$^{-1}$ indicates the C-O stretching vibration in Chitosan. The peaks at 850 and 1157 cm$^{-1}$ correspond to the saccharide structure. When two polymers are mixed, physical blends versus the chemical interactions are rejected by changes in the characteristic spectral bands in the typical spectrum of Chitosan/Starch composite.

**Fig. 8.** FTIR absorption spectra for, standard Chitosan, Starch, as well as their blends.

**Fig. 9.** FTIR absorption spectra for, prepared Chitosan, Cellulose, as well as their blends.

The amino peak of Chitosan was shifted from 1578 to 1584 cm$^{-1}$ with the addition of starch. This result indicates that interactions took place between the hydroxyl groups of Starch and the amino groups of Chitosan (Meenakshi et al., 2002).
The obtained FTIR spectrum of Chitosan/Starch composite suggested that the two forming components were compatible and an interaction existed between them.

Chitosan/Cellulose: The nature of mixing between Chitosan and Cellulose is of interest to the study. For this blending, if specific interactions took place, the most obvious and significant difference would be the appearance of new peaks or shift of existing peaks as in figures (9), (10); which represents the FTIR spectra of Chitosan, Cellulose and Chitosan/Cellulose blends. The FTIR spectrum of Chitosan shows peaks assigned to the Saccharide structure at 899 and 1153 cm\(^{-1}\), the amine group peak at around 1601 cm\(^{-1}\), \(N\)-acetylated chitin at 1655 cm\(^{-1}\) and the OH and NH peaks centered at 3418 cm\(^{-1}\). The FTIR spectrum of Cellulose shows peaks for the C=O functional groups at 1755 cm\(^{-1}\), the OH functional groups at 3528 cm\(^{-1}\), the CH\(_3\) groups at 1384 and 1249 cm\(^{-1}\), and the ether C–O–C functional groups at 1060 cm\(^{-1}\).

The FTIR spectra for the blends show that, the shift of the broad peak for OH and NH groups from 3528 to 3483 and to 3479 cm\(^{-1}\) with increasing Cellulose content of the blend, indicating the increased interaction of amine groups in Chitosan and OH groups in Cellulose. This is in a good agreement with that obtained by (Chunxiu Liu 2005).

![FTIR spectra](image)

**Fig. 10.** FTIR absorption spectra for, standard Chitosan, Cellulose, as well as their blends.

**References**


