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Preparation of chitosan and its miscibility studies with gelatin using viscosity, ultrasonic and refractive index

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Abstract

Chitosan was prepared from chitin by deacetylation using hot alkali (50% NaOH), and the DD (Degree of Deacetylation) was determined using FTIR spectroscopic method. The prepared chitosan was dissolved in buffer solution (0.2 M sodium acetate and 0.1 N acetic acid) and blended with modified natural polymer gelatin in solution. The miscibility of chitosan/gelatin blend in buffer solution was investigated by viscosity, ultrasonic and refractive index method at 30 and 50 °C. Using viscosity data, the interaction parameter μ and α, based on Chee and Sun et al. approaches were determined. The values indicated that the blend is immiscible in all proportion. The variation of ultrasonic and adiabatic compressibility with blend composition also supports the Sun et al. approach. This was further confirmed by refractive index method. The variation of temperature has no effect on miscibility of the chitosan/gelatin blend.

Keywords: FTIR; Chitosan; Gelatin; Miscibility; Blends

1. Introduction

Polymeric material has been closely associated in our daily life. In recent days natural biodegradable polymeric systems have gained importance due to the environmental pollution of non-biodegradable synthetic plastics. Chitin is the second most abundant naturally occurring polymer after cellulose and is found in the exoskeleton of crustaceans, in fungal cell wall and in other biological materials (Andrady & Xu, 1997). Chitosan is derived from chitin by deacetylation in the presence of hot alkali. Chitosan is a copolymer consisting of β-(1-4)-2-acetamido-D-glucose (20%) and β-(1-4)-2-amino-D-glucose (80%) units (Rudrapatnam, Tharanathan, & kittur, 2003). Chitosan described in terms of the degree of deacetylation and average molecular weight and their importance resides in their antimicrobial properties in conjunction with their cationicity and their film-forming properties (Muzzrali, 1996).Chitosan is a natural polysaccharide, generally considered as a safe, biocompatible and biodegradable material. During the past two decades, chitosan has been evaluated for numerous applications such as pharmaceutical, agricultural, food industry, and waste water treatment agent (Kumar, 2000). Higher molecular weight chitosan have been reported to have good film-forming properties as a result of intra and intermolecular hydrogen bonding (Muzzarelli & Peter, 1997). The chitosan film characteristics, however, varied from one report to another, differences in the sources of chitin used to produce chitosan, chitosan material properties, solvents used, methods of film preparations may affect the quality of the film.

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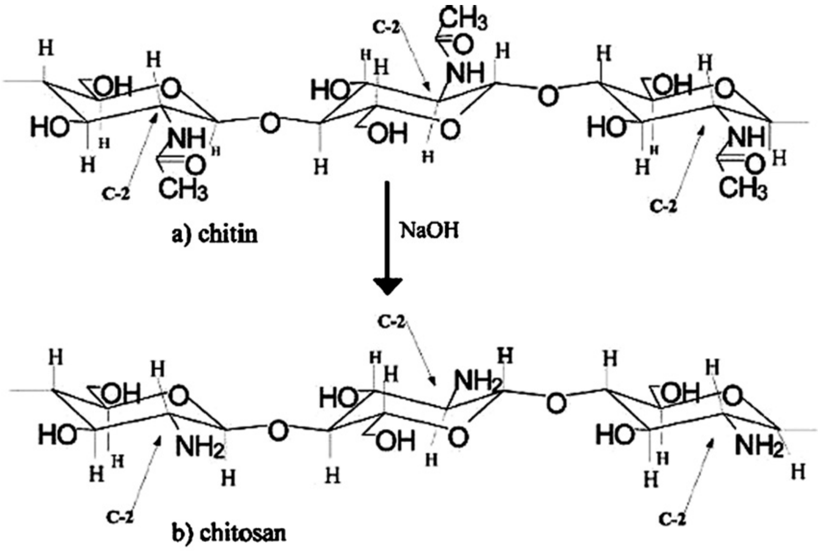

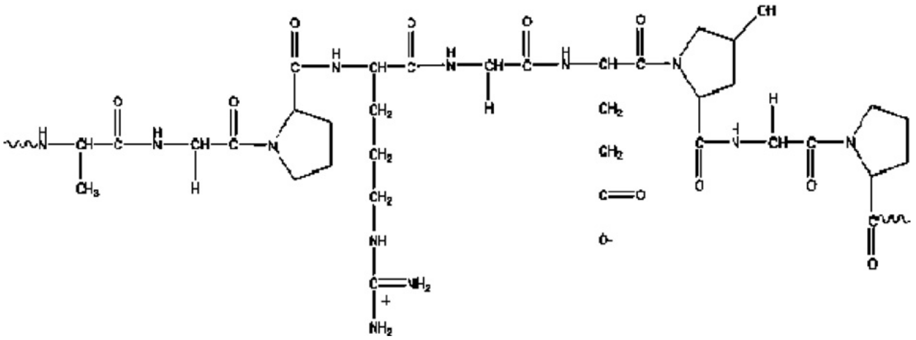

Blending of polymers is one of the simplest methods to obtain a variety of physical and chemical properties from the constituent polymer at a molecular level. The gain in newer properties depends on the degree of compatibility or miscibility of the polymer. There have been number of techniques to study the miscibility of polymer blends such as neutron scattering, inverse gas chromatography electron microscopy etc. (Olasisi, Robeson, & Sham, 1979). Most of the techniques may be complicated, costly and time consuming. Chee (1989) and Sun (Sun, Wang, & Feng, 1992) suggested viscometry to polymer–polymer miscibility. Paladhi and Singh (1994a, 1994b) showed that the variation of ultrasonic velocity and viscosity with blend composition is linear for miscible and non-linear for immiscible blends.

We have prepared chitosan from chitin by deacetylation using hot alkali; hence prepared chitosan was blended with gelatin in buffer solution (0.2 M sodium acetate and 0.1 N acetic acid). Both the polymers are modified natural polymer having numerous applications. Gelatin is a heterogeneous mixture of water-soluble proteins of high average molecular weight, substantially pure protein food ingredient, obtained by the thermal denaturation of collagen (Bailey & Paul, 1998). Like its parent protein, collagen, it is unique in that it contains 14% hydroxyproline, 16% proline, and 26% glycine (Baily & Light, 1989). Structurally gelatin contains a large number of glycine (almost 1 in 3 residues, arranged every third residue), proline and 4-hydroxyproline residues. A typical structure is -Ala-Gly-Pro-Arg-Gly-Glu-4Hyp-Gly-Pro-. Gelatin is soluble in hot water, used in different fields of applications as foaming agent protective colloid industry, in pharmaceutical industry etc.

2. Experimental

Chitin was received from Balaji aqua and agro products Pvt, ltd. Vijayawad, gelatin research grade from Qualigen, sodium acetate/glacial acetic acid buffer, and NaOH were used in this study. A dilute solution of 1% of homopolymer was prepared and the blend of chitosan/gelatin of different compositions 10/90, 20/80, 30/70, 40/60, 50/50, 60/40, 70/30, 80/20, and 90/10 were prepared in buffer solution. Viscosity, ultrasonic velocity and refractive index were determined at 30 and 50 °C. The required temperature was maintained in thermostat bath with a thermal stability of ±0.5 °C. Ultrasonic experimental cell has a double-walled jacket and thermo-stated water was circulated in it. The experimental frequency was 2 MHz and the velocity measurements were accurate to better than ±0.5%. The refractive indices of the blend solutions were measured with an Abbe's refractometer (Varadarajulu & Mabusab, 1998) and the accuracy of the refractive index measurements is ±0.02%.

3. Results and discussion

3.1. Preparation and characterization of chitosan

Chitin was dispersed in 50% w/w NaOH solution and refluxed at 110 °C for 5 h. The mixture was cooled to room temperature, filtered, and washed with water several times until the filtrate was neutral. The chitosan sample obtained was dried in an oven at 60 °C. The degree of deacetylation (DD) of chitosan was determined by FTIR method (Fig. 6) (Miya, Iwamoto, Yoshikawa, & Mima, 1980). The degree of deacetylation (DA) was determined by considering the OH band at 3450 cm^{-1} as a reference. The acetyl content (%) was defined from the ratio of absorbance: $(A1655\ cm^{-1}/A3450\ cm^{-1}) \times 100/1.33$. The degree of deacetylation (DD) was computed from %DA and it was found to be 60%. The viscosity average molecular weight of chitosan was determined by using Ostwald viscometer at 30 °C in 0.1 M acetic acid and 0.2 M sodium acetate buffer solution and was calculated by using Mark–Houwink's equation:

$$[\eta] = K \times [M]^{\alpha}$$

where, K and α constants for the buffer solution and chitosan system and their value is $K = 3.5 \times 10^{-4}$, and $\alpha = 0.76$, respectively.

The reduced viscosities for different concentration of chitosan were tabulated in Table 5. The $[\eta]$ from the graph = 4.8, the molecular weight of the prepared Chitosan was found to be 2.70 kDa.

Measured values of reduced viscosity data for chitosan, gelatin and their blend compositions 10/90, 20/80, 30/70, 40/60, 50/50, 60/40, 70/30, 80/20, and 90/10 at 30 and 50 °C are presented in Tables 1 and 2, respectively. Figs. 1 and 2 show the Huggins plots for the pure components and their blends at 30 and 50 °C, respectively (Fig. 3).

Chitosan is a high molecular weight viscous liquid. Hence, there is a slight positive deviation in the Huggins plots above 0.6% concentrations, Since Huggins plot is applicable only for dilute solutions, and hence data up to 0.6% concentrations is considered.

The evaluated values of the interaction parameter μ and α are presented in Table 3. It is clearly evident from Table 3 that, the interaction parameters μ and α values are negative for all the blend composition range at 30 and 50 °C. The negative values of μ and α indicate the immiscibility of the blend at both temperatures. However, in the presence of plasticizers like, glycerol, Sorbitol etc., the polymers are compatible and has good film-forming characteristics (Arvanitoyannis, Nakayama, & Aiba, 1998).

In order to confirm the correct nature of the blend under consideration, ultrasonic velocity and refractive index of the chitosan/gelatin blend of various compositions at different temperatures were measured and are given in Table 4.

In order to quantify the miscibility of the polymer blends, Chee (1989) suggested the general expression for

Table 1
Reduced viscosity data for chitosan/gelatin and their blends in buffer at 30 °C

Concentration (g/dL)	$\eta_{sp/C}$ (dL/g) at 30 °C									
	Chitosan	Gelatin	Chitosan/gelatin blend compositions							
			90/10	80/20	70/30	60/40	50/50	40/60	30/70	20/80	10/90
0.60	19.66	0.29	10.00	8.35	7.48	6.21	4.66	3.50	2.59	1.61	0.90
0.50	16.83	0.28	8.43	7.19	6.45	5.43	4.19	3.21	2.42	1.50	0.90
0.40	14.50	0.28	7.47	6.40	5.83	4.97	3.90	3.00	2.29	1.43	0.89
0.30	11.65	0.29	6.67	5.71	5.24	4.52	3.61	2.86	2.22	1.39	0.67
0.20	9.46	0.28	5.77	5.00	4.64	4.11	3.39	2.68	2.14	1.13	0.41
0.10	7.44	0.26	4.88	4.40	4.05	3.93	3.21	2.62	2.14	1.19	0.47
0.00	4.82	0.25	4.1	3.20	3.50	3.40	3.00	2.40	2.00	1.00	0.30

Table 2
Reduced viscosity data for the chitosan/gelatin and their blends at 50 °C

Concentration (g/dL)	$\eta_{sp/C}$ (dL/g) at 50 °C									
	Chitosan	Gelatin	Chitosan/gelatin blend compositions							
			90/10	80/20	70/30	60/40	50/50	40/60	30/70	20/80	10/90
0.60	12.30	0.20	7.04	5.69	6.05	4.44	3.44	2.43	1.82	1.27	0.61
0.50	10.82	0.195	6.09	5.08	5.21	3.97	3.08	2.19	1.80	1.21	0.57
0.40	9.21	0.19	5.48	4.69	4.60	3.65	2.86	2.10	1.75	1.15	0.565
0.30	8.04	0.165	4.76	4.23	4.17	3.39	2.70	2.01	1.65	1.13	0.56
0.20	6.59	0.16	4.21	3.65	3.25	3.01	2.46	1.90	1.60	1.12	0.55
0.10	5.24	0.15	3.65	3.17	2.86	2.86	2.38	1.75	1.59	1.10	0.48
0.0	4.00	0.14	3.00	2.70	2.60	2.40	2.20	1.60	1.50	1.00	0.40

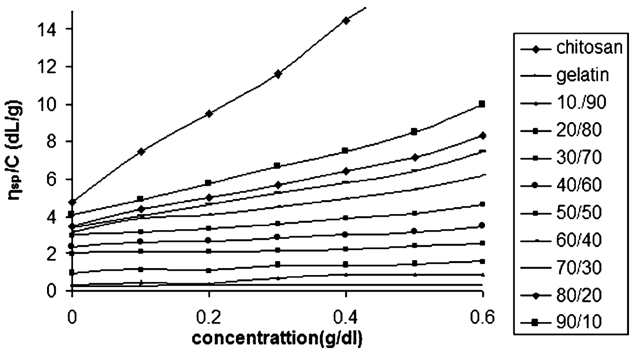

Fig. 1. Huggins plot for 1% w/v, chitosan/gelatin blend in buffer solution at 30 °C.

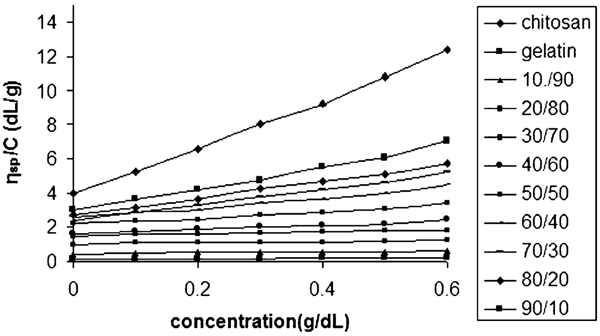

Fig. 2. Huggins plot for 1% w/v, chitosan/gelatin blend in buffer solution at 50 °C.

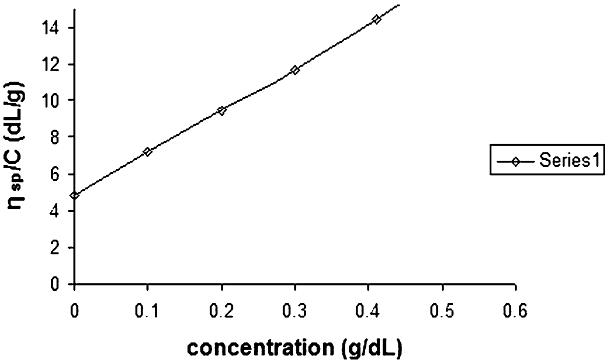

Fig. 3. Plot of reduced viscosity vs. concentration at 30 °C.

Table 3
The interaction parameters μ and α of 1% w/v, chitosan/gelatin blend at 30 and 50 °C

Composition of chitosan/gelatin	At 30 °C		At 50 °C	
	μ	α	μ	α
10/90	−0.23	−3.20	−0.41	−2.38
20/80	−0.26	−0.86	−0.43	−4.33
30/70	−0.26	0.98	−0.42	−2.76
40/60	−0.24	−0.63	−0.45	−1.09
50/50	−0.22	−0.48	−1.31	−0.96
60/40	−0.08	−0.32	−0.49	−3.18
70/30	−0.11	−0.15	−0.48	−2.59
80/20	−0.08	−0.10	−0.08	−0.29
90/10	−0.04	−0.06	−0.04	−0.37

Table 4
Ultrasonic and refractive index at 30 and 50 °C

Composition of chitosan/gelatin	Ultrasonic velocity (m/s)		Refractive index	
	At 30 °C	At 50 °C	At 30 °C	At 50 °C
0/100	1470.40	1729.00	1.3450	1.3450
10/90	1608.00	1589.00	1.3455	1.3445
20/80	1466.80	1465.80	1.3460	1.3450
30/70	1200.60	1598.00	1.3465	1.3440
40/60	1468.00	1469.60	1.3455	1.3440
50/50	1732.00	1596.80	1.3450	1.3445
60/40	1736.80	1466.20	1.3460	1.3440
70/30	1599.80	1607.60	1.3450	1.3445
80/20	1859.80	1599.60	1.3460	1.3450
90/10	2136.20	1468.00	1.3455	1.3450

Table 5
Reduced viscosity data for molecular wt determination at 30 °C

Concentration (g/dL)	$\eta_{sp/c}$ (dL/g)
0.60	19.66
0.50	16.83
0.40	14.5
0.30	11.65
0.20	9.46
0.10	7.44

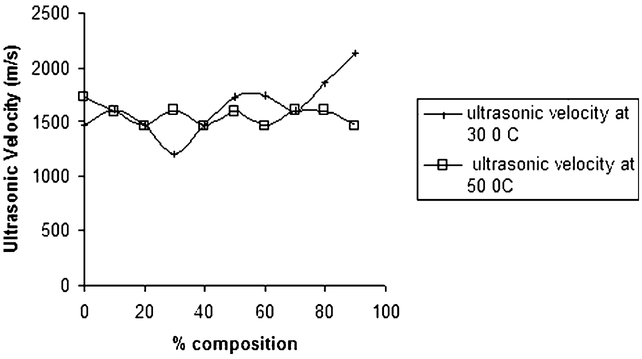

Fig. 4. Ultrasonic velocity of blend at 30 and 50 °C.

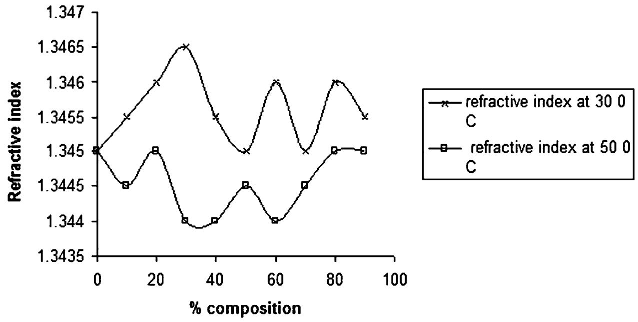

Fig. 5. Refractive index of blend at 30 and 50 °C.

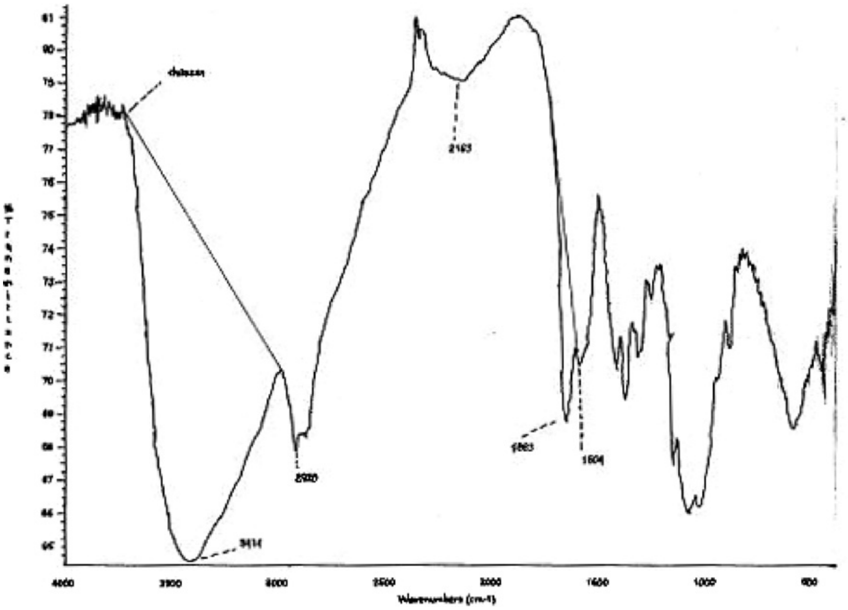

Fig. 6. FTIR of prepared chitosan.

the interaction parameter when the polymers are mixed in weight fraction W_1 and W_2 as

$$\Delta B = \frac{b - \bar{b}}{2W_1W_2} \tag{1}$$

where $\bar{b} = W_1b_{11} + W_2b_{22}$, in which b_{11} and b_{22} are the slopes of the viscosity curves for the components and b is related to Huggins coefficient K_H as

$$b = K_H[\eta]^2 \tag{2}$$

for tertiary system, it is also given by

$$b = W_1^2b_{11} + W_2^2b_{22} + 2W_1W_2b_{12} \tag{3}$$

where b_{12} is slope for the blend solution.

However, Chee's theory fails because experimental data are in conflict with theoretical predictions (Chee, 1989). In such cases Chee has defined a more effective parameter to predict the compatibility:

$$\mu = \frac{\Delta B}{\{[\eta]_2 - [\eta]_1\}^2} \tag{4}$$

where $[\eta]_1$ and $[\eta]_2$ are the intrinsic viscosities for the pure component solutions. The polymer blend is miscible if $\mu \geqslant 0$ and immiscible when $\mu < 0$ Recently Sun (Sun et al., 1992) suggested a most satisfactory new equation for the determination of polymer miscibility as

$$\alpha = K_m - \frac{K[\eta]_1^2W_1^2 + K_2[\eta]_2^2W_2^2 + 2\sqrt{K_1K_2[\eta]_1[\eta]_2W_1W_2}}{\{[\eta]_1W_1 + [\eta]_2W_2\}^2} \tag{5}$$

where K_1, K_2 and K_m are the Huggins constants for individual components 1, 2 and blend, respectively. The long-range hydrodynamic interactions are considered while deriving the equation. The polymer blend is miscible if $\alpha \geqslant 0$ and immiscible when $\alpha < 0$.

The variations of ultrasonic and refractive index, with blend compositions are given in Fig. 4 and 5 . The graphs show only non-linear regions over the entire composition range. It was already established that the variation is linear for miscible and non-linear for immiscible blends.

4. Conclusion

Based on viscosity, ultrasonic velocity and refractive index measurements, it is concluded that the chitosan/gelatin blend has been found to be immiscible in all compositions and there is no effect of temperature on miscibility of the blend. The reason may be due to the complex structure of both chitosan and gelatin and also the secondary force, which is responsible for compatibility of two polymers, may not be sufficient enough to over come the repulsion between the NH_2 groups present on the both the polymers. The non-linearity of gelatin also may be the one of the criteria for immiscibility with chitosan.

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