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Chitosan and gelatin based edible films: state diagrams, mechanical and permeation properties

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Abstract

Films of chitosan and gelatin were prepared by casting their aqueous solutions (pH ≈ 4.0) at 60°C and evaporating at 22 or 60°C (low- and high-temperature methods, respectively). The physical (thermal, mechanical and gas/water permeation) properties of these composite films, plasticized with water or polyols, were studied. An increase in the total plasticizer content resulted in a considerable decrease of elasticity modulus and tensile strength (up to 50% of the original values when 30% plasticizer was added), whereas the percentage elongation increased (up to 150% compared to the original values). The low-temperature preparation method led to the development of a higher percentage renaturation (crystallinity) of gelatin which resulted in a decrease, by one or two orders of magnitude, of CO_2 and O_2 permeability in the chitosan/gelatin blends. An increase in the total plasticizer content (water, polyols) of these blends was found to be proportional to an increase in their gas permeability. © 1998 Elsevier Science Ltd. All rights reserved.

Keywords: Gas permeability; Edible films; Chitosan; Gelatin; Polyols; Glycerol; Tensile strength; State diagrams

1. Introduction

Chitin is the second most abundant naturally occurring biopolymer (after cellulose) and is found in the exoskeleton of crustaceans, in fungal cell walls and in other biological materials (Andrady and Xu, 1997). It is mainly poly(β-(1-4)-2-acetamido-D-glucose), which is structurally identical to cellulose except that a secondary hydroxyl on the second carbon atom of the hexose repeat unit is replaced by an acetamide group. Chitosan is derived from chitin by deacetylation in the presence of alkali. Therefore, chitosan is a copolymer consisting of β-(1-4)-2-acetamido-D-glucose and β-(1-4)-2-amino-D-glucose units with the latter usually exceeding 80%. Chitosans are 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 (Muzzarelli, 1996). Film-making conditions, including solvent pH, ionic strength, type of solvent (acid) used and annealing treatment, are parameters often manipulated to alter the mechanical properties and membrane porosity. Ionic strength or pH can be manipulated in order to reduce inter- and intramolecular electrostatic repulsion between chitosan chains, thus allowing the chains to approach each other and enhance the inter- and intramolecular hydrogen bonding (Chen et al., 1994). Chitosan has been extensively used over a wide range of applications, such as a biomaterial in medicine either on its own or as a blend component (Zhang et al., 1997; Hasegawa et al., 1992a, b, 1994), a membrane filter for water treatment (Kawamura, 1995; Muzzarelli, 1977), a biodegradable, edible coating or film in food packaging (Wong et al., 1992; El Ghaouth et al., 1991a, b; Butler et al., 1996) a dietary fibre, and a medicine against hypertension because of its scavenging action for chloride ions (Okuda, 1995; Furda and Brine, 1990; Muzzarelli, 1996).

It is usually little appreciated that collagen constitutes as much as 30% of total human protein with similar proportions being found in most animals. It is widespread in both vertebrates and invertebrates from primitive marine worms to mammals, providing strength and support for the animals' tissues and organs. The collagen molecule exists as a triple helix, comprising three discrete α-chains (three-dimensional structure), which leaves space for interchain hydrogen bonding. The existing imino acids impart rigidity to the molecule and the interstitial water molecules might act as hydrogen-bond bridges, thus contributing to the

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stability of the helix. The three α-chains of collagen are not identical but have slight variations in their imino acid content (Johnston-Banks, 1990). To convert insoluble collagen into soluble gelatin, the primary structure of which closely resembles that of the parent collagen, acid or alkaline pretreatments are required for cleaving a sufficient number of covalent cross-links in the collagen. Partial removal of amide groups results in increase of carboxyl groups in the gelatin molecule, thus lowering the isoelectric point.

The three-dimensional gel network of gelatin is composed of microcrystallites interconnected with amorphous regions of randomly coiled segments (Slade and Levine, 1987). Gelatin's ability to form thermoreversible gels with a melting point close to body temperature (Achet and He, 1995) has contributed substantially to an increase in its applications. Gelatin's largest single food use is in gel desserts because of the unique 'melt at mouth temperature' (Slade and Levine, 1987; Kalafatas et al., 1975; Johnston-Banks, 1990), in frozen foods and in dairy products as a protective colloid or stabilizer, i.e. ice crystal inhibitor (Fiscella, 1983; Morley, 1984). Gelatin has also been used in photographic emulsions, playing a multipurpose role such as a protective colloid, ripening agent and binder (Jolley, 1970), in the textile industry as an adhesive (Bradbury and Martin, 1952) and in the pharmaceutical industry for the production of tablets and hard capsules (Healey et al., 1974; Johnson, 1965). Food coating and casing applications such as sausage casings and poultry coatings, with or without the presence of antimicrobial compounds, are envisaged as another important and promising issue which has primarily received attention by the meat industry (Keil and Hills, 1961; Keil et al., 1960; Hood, 1987; Moorjani et al., 1978; Klose et al., 1952). Occasionally, gelatin has been used in conjunction with other hydrocolloids such as acacia (gum arabic), alginate and pectate esters, soluble and hydroxy propyl starch (Deasy, 1984; McKay et al., 1985; Arvanitoyannis et al., 1997a, b). Blending of chitosan with other hydrophilic polymers such as poly(vinyl alcohol), poly(vinyl pyrrolidone) or pectin, occasionally followed by alkali cross-linking, has been suggested as a promising avenue for the production of 'tailor-made' blends (Blair et al., 1987; Quarashi et al., 1992; Yao et al., 1996; Andrady, 1992; Suto and Ui, 1996).

The aim of this article is to investigate the properties of binary or ternary/quartenary single-phase blends consisting of a protein–polysaccharide matrix plasticized with water and/or other polyols and to correlate these results with those reported in the literature for materials of similar resources.

2. Experimental

2.1. Materials

Chitosan was purchased from Seigakaku Corporation, Tokyo, Japan (pH ≈ 6.8–7.0, $M_n = 1.5 \times 10^5$, $M_w = 6.4 \times 10^5$). Gelatin from pigskin (pH ≈ 4.0–4.5, $M_n = 4.2 \times 10^5$, $M_w = 1.3 \times 10^6$, practical grade), glycerol, sorbitol and sucrose (analytically pure) were purchased from Wako Chemicals, Japan.

2.2. Preparation procedure for permeability and tensile measurements

Films of thickness 0.8 ± 0.06 mm were prepared by casting 5% aqueous gelatin and 2% aqueous chitosan solutions in 2% acetic acid, after previous mixing at pH ≈ 4.0 and 22°C or 60°C, on metal trays, followed by evaporation of water. The thickness of each sample was measured at eight different points with a micrometer and the average was taken. At 60°C the evaporation was completed within 6 h. A template was used to cut testing strips from the films so that the testing section measured 50 × 10 mm. In order to examine the physical properties of films as a function of moisture content, films were placed in relative humidity chambers over salt solutions or phosphorus pentoxide and the moisture content was determined by drying to constant weight (Arvanitoyannis et al., 1994, 1996).

2.3. Preparation of samples for dynamic mechanical thermal analysis (DMTA) and three-point bend measurements

Preparation of thicker samples of chitosan/gelatin was carried out by repeated evaporation of chitosan/gelatin solutions in 2-cm-deep stainless steel rectangular frames (20 cm × 10 cm × 2 cm) at a temperature of 22 or 60°C. Conditioning of samples at different relative humidities was carried out as described previously (Arvanitoyannis et al., 1994).

2.4. Dynamic mechanical thermal analysis (DMTA) measurements

A dynamic mechanical thermal analyser (DMTA, Mark II, Polymer Laboratories, UK) with a heating rate of $2°\mathrm{C\ min}^{-1}$ and a single cantilever bending mode at 1 Hz was calibrated each day and measurements were taken using triplicate samples.

The glass transition was defined as the midpoint between the onset of the drop in the elastic modulus $\Delta E'$ (obtained from the intercept of the 'glassy' baseline and the tangent to the point of the steepest drop in modulus) and the peak in tan δ ($\tan \delta = E''/E'$, where E'' is the loss modulus).

2.5. Differential thermal analysis (DTA) measurements

DTA measurements were taken using a Shimadzu differential thermal analyser equipped with a liquid N_2 cooling accessory and connected to an IBM/PC and a Hewlett-Packard plotter. Hermetically sealed aluminium pans were used with an empty aluminium pan as reference. The sample size for DTA was approximately 10–20 mg. Temperature calibration was carried out with cyclohexane, dodecane and octane. Heat flow calibration was carried out with reference to the known melting enthalpy of indium metal (purity 99.9%) from Goodfellows Metals. The purge gases used were dry helium and dry nitrogen. The T_gs were determined from the second run after heating, quenching with liquid nitrogen and reheating at a heating rate of $10°C\ min^{-1}$. The percentage crystallinity with DTA was calculated according to Achet and He (1995). Data analysis to fit experimental values of T_g to the empirical Gordon–Taylor equation (Gordon and Taylor, 1952) was performed using TableCurve™ software (Jandel Scientific), a non-linear least-squares-fitting package:

$$T_g = \frac{w_1 T_{g_1} + k w_2 T_{g_2}}{w_1 + k w_2} \quad (1)$$

where w_1 and w_2 are the respective weight fractions of the polymer blend and water, T_{g_1} is the T_g of the composite polymer matrix, T_{g_2} is the T_g of the amorphous water, and k is a constant related to the strength of polymer–diluent interaction (the larger the k, the greater the plasticization effect). The k constant is equivalent to the ratio of the heat capacity changes at T_g of the pure diluent and the polymer matrix, $\Delta C_{p_1}/\Delta C_{p_2}$. A T_g of −135°C was used for water (Johari et al., 1987). Two plots are presented: one based on the best data fitting (optimization for both parameters, k and $T_{g_{matrix}}$), and the other based on a k value of 4 (an approximate estimate of $\Delta C_{p_1}/\Delta C_{p_2}$, based on literature values of ΔC_p for water and chitosan/gelatin, dotted line).

2.6. Measurements of gas permeability and water vapour permeability (WVP)

The measurements of gas permeability were carried out using a Davenport apparatus connected to an IBM/PC in accordance with ASTM D1434-66 (ASTM, 1966). Thickness was measured with a micrometer at 8 or 10 locations of the film.

Permeability (P) is the product of solubility (S) and diffusivity (D) according to the following equation:

$$P = D \cdot S \quad (2)$$

Assuming that unidirectional diffusion through a flat membrane occurs, diffusion can be expressed as follows:

$$J_i = -D_{i(ci)} \frac{dc_i}{dx} \quad (3)$$

where J is the flux, $D_{i(ci)}$ signifies that the diffusion coefficient is dependent on the composition of penetrant and c refers to the local gas or penetrant concentration.

The formula for the determination of the diffusion constant is as follows:

$$D = \frac{d^2}{6\theta} \quad (4)$$

where d is the thickness of the film and θ is the time lag for permeation. The lag is related to the time required by the gas to establish an equilibrium in an originally gas-free film. The extrapolation of the pressure increase–time curve to the zero axis will produce the time lag (θ) (Amerongen, 1947, 1949). The quantity of gas (Q) that will then pass through the film is directly proportional to the difference in the pressure exerted by the gas on each face of the film ($p_1 - p_2$) and is inversely proportional to the thickness (x). It is also directly proportional to the area exposed (A) and the time (t) for which permeation occurs. Overall, the relationship can be expressed by the following equation:

$$Q = \frac{PAt(p_1 - p_2)}{x} \quad (5)$$

where P has a constant value for a specific combination of gas and polymer at a given temperature and is variously known as the transmission factor or permeability factor/constant/coefficient (van Krevelen, 1990).

Water vapour permeability (WVP) measurements were carried out as reported previously (Martin-Polo et al., 1992).

2.7. Mechanical properties

2.7.1. Tensile strength and percentage elongation

Tensile strength and percentage elongation were measured on testing strips, after their equilibration at various relative humidities, using an Instron Universal Testing Instrument (model 1122) operated according to ASTM D828-88 (ASTM, 1989). Measurement conditions and calculations of tensile strength and percentage elongation were made as described previously (Arvanitoyannis and Psomiadou, 1994).

2.7.2. Three-point bending test

All samples were cut with a scalpel, scissors or saw into bars about 30 × 8 × 2 mm and stored for at least 3 weeks over saturated salt solutions at room temperature in order to obtain various water contents which were measured on three replicates by drying at 130°C for 1 h. Three-point bend tests were carried out at room temperature using an Instron texturometer at a cross-head speed of $50\ mm\ s^{-1}$ on samples previously equilibrated at various relative humidities. The initial slope of the force/distance graph and the sample dimensions were used to calculate the Young's modulus. The peak force was also recorded.

3. Results and discussion

3.1. Thermal and thermal mechanical properties

The addition of low-molecular-weight compounds (polyols or water) to chitosan/gelatin blends was shown to lower both the melting (T_m) and transition point (T_g) proportionally to the plasticizer content of the blend (Tables 1 and 2,

Table 1
Thermal and thermomechanical properties of chitosan/gelatin(1:1)/glycerol, chitosan/gelatin(1:1)/sorbitol blends, prepared with low- and high-temperature processes. The results give the average and the standard deviation of at least three or five measurements for thermal and permeability measurements, respectively

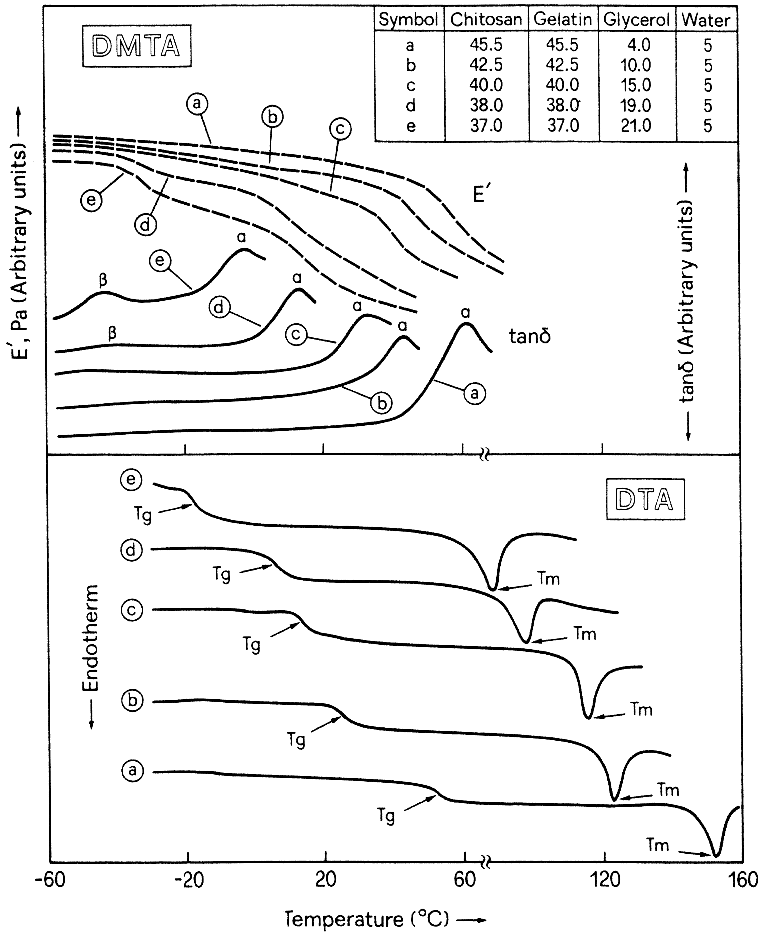
				DTA			T_g			
				T_m	ΔH (J g^{-1})	%Rn[a]	DTA	DMTA (E′)	DMTA (tan δ)	Permeability (inflection)
Low-temperature process										
Chitosan	Gelatin	Glycerol	Water							
47.5	47.5	0	5	153.4 ± 2.9	27.6 ± 1.8	89.0	81.2 ± 4.2	84.6 ± 2.9	90.8 ± 2.9	82.0 ± 3.5
45.5	45.5	4	5	146.3 ± 1.8	23.5 ± 1.9	79.1	57.4 ± 3.6	67.0 ± 2.5	70.9 ± 2.3	66.4 ± 3.9
40.0	40.0	15	5	138.7 ± 2.5	19.8 ± 0.8	75.8	21.7 ± 2.5	24.5 ± 1.9	30.0 ± 3.5	31.3 ± 2.6
Chitosan	Gelatin	Sorbitol	Water							
45.5	45.5	4	5	146.1 ± 1.5	25.2 ± 1.8	84.8	51.9 ± 3.9	56.5 ± 2.8	62.5 ± 2.1	55.8 ± 4.8
40.5	40.5	14	5	137.3 ± 2.1	18.9 ± 1.2	71.4	17.5 ± 1.8	23.3 ± 1.9	35.0 ± 3.9	22.1 ± 3.6
High-temperature process										
Chitosan	Gelatin	Glycerol	Water							
47.5	47.5	0	5	148.5 ± 2.6	23.3 ± 1.9	75.1	71.2 ± 4.7	75.3 ± 1.9	63.4 ± 1.8	70.5 ± 2.2
45.0	45.0	5	5	140.0 ± 1.9	18.9 ± 2.0	64.3	39.6 ± 3.5	48.8 ± 1.6	55.8 ± 2.4	62.3 ± 2.8
39.5	39.5	16	5	134.3 ± 2.0	15.0 ± 1.2	58.1	10.3 ± 1.5	14.5 ± 1.8	20.3 ± 2.2	–
Chitosan	Gelatin	Sorbitol	Water							
44.5	44.5	6	5	136.4 ± 2.5	16.7 ± 1.5	57.5	34.9 ± 2.9	38.6 ± 1.9	43.5 ± 3.8	36.5 ± 1.9
40.0	40.0	15	5	127.6 ± 3.1	13.0 ± 1.2	49.8	8.8 ± 2.5	13.7 ± 2.4	21.2 ± 1.5	–

[a] Percentage renaturation calculated from $\Delta H_{\text{gelatin in blend}}/\Delta H_{\text{collagen}} \times 100$.

Figs. 1 and 2). The recorded decrease in percentage crystallinity in chitosan/gelatin blends may be attributed to the incorporation of chitosan in the gelatin network, similarly to what was previously observed for chitosan/poly(vinyl alcohol) blends (Arvanitoyannis et al., 1997c; Miya et al., 1983, 1984). The dilution of gelatin by chitosan is the main reason for the observed decrease in crystallization. It is worth mentioning that no phase separation was evident in the plasticized composite matrix for up to 25% total plasticizer content (TPC). Phase separation was not encouraged because of the hydrophilic nature of gelatin which gave rise to hydrogen bonds between gelatin and water, and gelatin and polyol. Although gelatin's thermogram shows very clear T_g and T_m, in chitosan's trace there is no obvious transition. The chitosan/gelatin blends show endothermic peaks of an intensity proportional to the plasticizer content. High plasticizer contents were related to lower crystallinities and lower T_m and T_g values (Tables 1

Table 2
Glass transition temperatures (T_g, °C) of blends of gelatin and soluble starch conditioned at different relative humidities

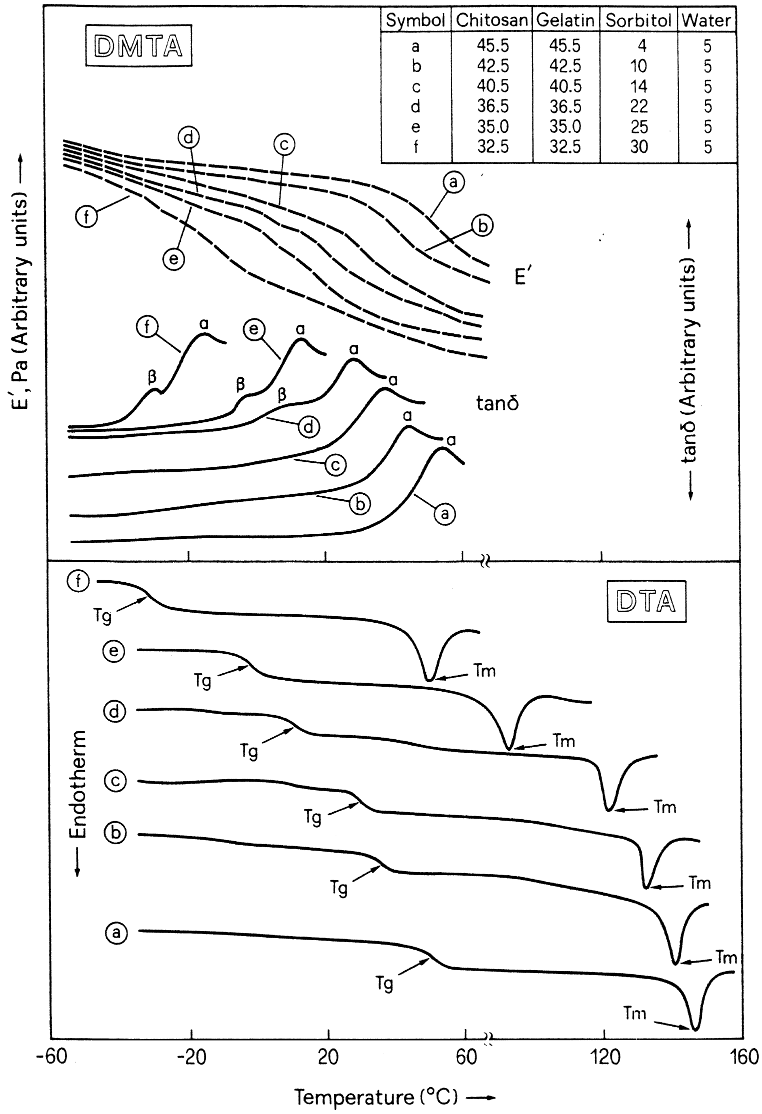
Chitosan %	Water %	Gelatin%	DTA (2nd run)	DMTA (E′)	DMTA (tan δ)	Permeability (inflection)
Low-temperature process						
–	10	90	55.7 ± 5.5	58.3 ± 4.9	66.9 ± 2.1	51 ± 2
5	10	85	58.1 ± 3.0	60.1 ± 2.5	74.1 ± 4.8	55 ± 2
8	10	82	60.0 ± 1.8	63.0 ± 1.8	76.5 ± 3.8	59 ± 3
15	10	75	61.5 ± 2.5	68.2 ± 2.8	77.7 ± 2.0	63 ± 4
22	10	68	66.1 ± 3.9	72.6 ± 3.9	79.5 ± 2.6	64 ± 3
25	10	65	69.0 ± 2.8	75.1 ± 2.3	81.0 ± 3.5	70 ± 3
48	4.0	48	80.2 ± 2.0	78.0 ± 2.5	85.2 ± 2.7	75 ± 2
46	8.0	46	67.5 ± 5.9	69.1 ± 2.2	76.4 ± 2.9	65 ± 3
43	14.0	43	51.6 ± 3.6	58.4 ± 3.9	65.2 ± 2.6	50 ± 2
39	22.0	39	32.0 ± 2.5	35.3 ± 3.8	40.7 ± 2.8	34 ± 3
High-temperature process						
–	10	90	52.0 ± 1.9	56.3 ± 1.9	63.1 ± 3.2	48 ± 3
5	10	85	53.5 ± 2.8	59.2 ± 2.4	66.4 ± 4.9	52 ± 2
10	10	80	56.0 ± 2.5	61.0 ± 2.5	68.0 ± 2.5	54 ± 3
15	10	75	57.4 ± 4.6	62.5 ± 2.8	71.3 ± 3.5	57 ± 2
22	10	68	58.5 ± 2.7	64.4 ± 3.6	74.0 ± 2.7	60 ± 2
28	10	62	60.0 ± 2.5	66.5 ± 2.6	75.1 ± 2.4	61 ± 3
48	4	48	63.2 ± 2.2	68.6 ± 3.0	79.6 ± 2.5	64 ± 4
46	8	46	56.0 ± 1.9	59.5 ± 2.5	67.0 ± 2.2	58 ± 2
42.5	15	42.5	35.4 ± 2.8	41.0 ± 2.3	49.4 ± 2.9	41 ± 2



Symbol	Chitosan	Gelatin	Glycerol	Water
a	45.5	45.5	4.0	5
b	42.5	42.5	10.0	5
c	40.0	40.0	15.0	5
d	38.0	38.0	19.0	5
e	37.0	37.0	21.0	5

Fig. 1. Representative traces of chitosan/gelatin/water/glycerol blends prepared by the low temperature process: DTA and DMTA.

and 2, Fig. 1). Gelatin consists of crystalline and amorphous regions randomly interspersed within the composite polymer matrix. The cohesion of chitosan/gelatin blends increases in the presence of several self-associated molecules, such as water and polyols, provided they do not exceed 20%. At a fixed moisture content the transition temperature (T_g) for the α-peak of chitosan/gelatin blends was found to decrease proportionally to an increase in the plasticizer content. No β-transition was observed if the TPC did not exceed 25%. Above 25% TPC the β-transition appeared at around –45°C with varying intensity depending on the TPC (Fig. 1). In Figs. 1 and 2 the storage modulus (E') is explicitly shown to go through the glassy, leathery and rubbery states for chitosan/gelatin blends plasticized with glycerol and sorbitol, respectively. The main difference is that in the latter the β-transition looks more like a ‘shoulder’ (partial overlapping of β- and α-transition peaks for tan δ), whereas in glycerol plasticized blends it is clearly differentiated. Furthermore, an increase in sorbitol content ($> 30\%$) did not result in a significant change of the β-peak with temperature, whereas the observed increase in its peak intensity should be attributed to enhanced molecular mobility. The plasticization induced by the water present in the glycerol and sorbitol plasticized blends should also be taken into account. In fact, water acts as a plasticizer of the other two low-molecular-weight compounds; glycerol and sorbitol. Since glycerol and sorbitol are more hydrophilic than the chitosan/gelatin matrix, it is only fair to assume that after conditioning at varying relative humidities the sorbed water amount is directly proportional to the glycerol/sorbitol content. The state diagram of the binary system composed of chitosan/gelatin (1:1) based on experimental data obtained from DTA is shown in Fig. 3a. The respective diagrams of the ternary systems (chitosan/gelatin/polyol 45/45/10 and 40/40/20 w/w/w) are given in Fig. 3b–e. The plasticizing effect of sorbitol was more pronounced than that of glycerol as in previous findings (Arvanitoyannis et al., 1996, 1997a, b, c; Psomiadou et al., 1996). T_g estimates for the dry binary/ternary polymer blends were obtained by using Gordon–Taylor plots (Gordon and Taylor, 1952) as shown in Fig. 3. The T_g of about 421 K for the dry chitosan/gelatin blend is decreased to 359 and 350 K for blends with 10 and 20% glycerol content and to 346 and 334 K for blends containing 10 and 20% sorbitol, respectively. When a k value of 4.0 was employed, the resulting Gordon–Taylor plots were close to the optimum fit in describing the T_g depression ($r^2 = 0.92$–0.95), thereby giving considerably higher T_g estimates for the dry polymer



Symbol	Chitosan	Gelatin	Sorbitol	Water
a	45.5	45.5	4	5
b	42.5	42.5	10	5
c	40.5	40.5	14	5
d	36.5	36.5	22	5
e	35.0	35.0	25	5
f	32.5	32.5	30	5

Fig. 2. Representative traces of chitosan/gelatin/water/sorbitol blends prepared by the low temperature process: DTA and DMTA.

matrix. Although the quaternary system is characterized by several interactions among the various components such as chitosan/gelatin–water or chitosan/gelatin–polyol, polyol–polyol and water–polyol (Tolstoguzow et al., 1985; Tolstoguzow, 1994), its plasticization is obvious not only in terms of its thermal properties (T_g and T_m lowering) but also in terms of its textural properties. The percentage renaturation of gelatin in chitosan/gelatin blends, given in Table 1, was calculated as follows:

$$\%R_n = \Delta H_m/\Delta H_{100\%\mathrm{renatured}} = \Delta H_m/\Delta H_{\mathrm{collagen}} \quad (6)$$

where $\Delta H_{\mathrm{collagen}} = 62.05\ \mathrm{J\ g^{-1}}$ (Achet and He, 1995; Macsuga, 1972).

Although the appropriateness of the terms ‘melting’ and ‘renaturation’ have been questioned repeatedly with regard to the collagen structural order, the terms melting, helix–coil transition and denaturation are still used interchangeably (Marshall and Petrie, 1980). The hot dried binary chitosan/gelatin films were initially amorphous and consisted mainly of single gelatin chains. Their formation is directly linked to water evaporation during which the random nature of the gelatin molecules in solution persists until the viscosity becomes too high for ordering to occur and the random configuration is instantaneously frozen. However, by conditioning this binary polymer system at various relative humidities, the system becomes endowed with increased mobility resulting in a new type of network where single-chain gelatin molecules are put together by triple stranded crystallites which are randomly aligned in the plane of the film. These crystallites act as mobile and non-permanent cross-links since they make the formation of a semicrystalline network feasible. The determination of percentage renaturation (Eq. (6)), a term equivalent to the crystallinity of gelatin, refers to the gradual conversion of the gelatin strands to a collagen-type triple helix. The gelatin renaturation is believed to occur through a multistep

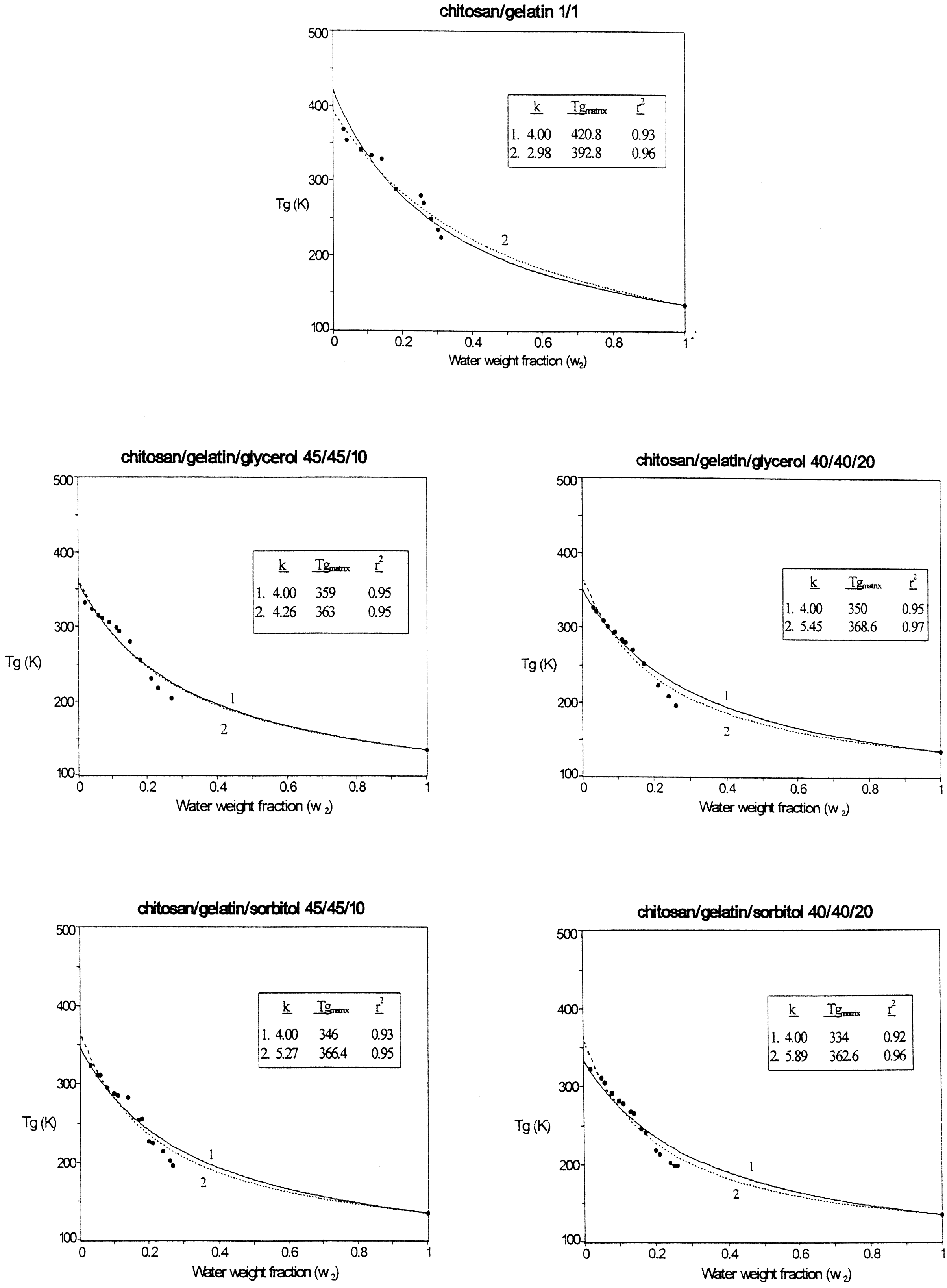


Fig. 3. State diagrams of chitosan/gelatin(1:1)/polyol blends. T_g versus water content (●), experimental data; solid and dotted lines give the corresponding Gordon Taylor plots of the data for different *k* values). Numbers in parentheses refer to weight fraction ratios of blend components.

mechanism; a randomly dispersed and disordered coil is converted to a type II helix, followed by an intermediate type I stage and, finally, the growth stage resulting in a collagen-type structure (Achet and He, 1995; Arvanitoyannis et al., 1997a, b, c). Table 1 shows that the helix–coil required enthalpy is directly proportional to the water content in the polymer matrix. It is important, however, that this moisture content does not exceed a certain 'threshold', about 15% (Arvanitoyannis et al., 1997a, b, c) because, in that case, the high mobility imparted to the system's components increases the extent of interactions especially among the minor and low-molecular-weight components (i.e. water, polyols), finally resulting in disturbance or inhibition of the renaturation process.

3.2. Mechanical properties

3.2.1. Tensile strength and percentage elongation

Table 3 shows the results obtained from the mechanical measurements of chitosan/gelatin blends after their conditioning in environments of various relative humidities. To get a better insight of the behaviour of films in terms of tensile strength and elongation, a more detailed knowledge of these blends' morphology is required. Gelation occurs in the chitosan/gelatin solution via the formation of triple-stranded crystallites whose size, number and perfection greatly depends upon the thermal history of the gel. In fact, it was shown elsewhere that the lower the gelation temperature, the more crystalline the gel is, but the smaller and less perfect are its crystallites (Bradbury and Martin, 1952; Jolley, 1970; Arvanitoyannis et al., 1997a, b, c). Drying the gelled film results in a substantial water loss. This volume reduction results in distortion of the isotropic gel network thereby leading to squashing of the crystallites and stretching of single molecules (Jolley, 1970). Table 3 shows that the films obtained by evaporation at high temperature (≈60°C) are weaker, exhibiting lower tensile strength and elongation than those prepared by the low-temperature process, for TPC up to 25%. When the TPC is higher than 25% a considerable increase in percentage elongation is observed which is greater for the less-crystalline films (high-temperature process). Although this result may appear, initially, not to conform with the polymer theories, it is essential to understand the role of water. At high relative humidities (>65%) each gelatin molecule is plasticized by a monolayer of water. It is reasonable to suggest that the less-crystalline chitosan/gelatin films (high-temperature process) are more readily plasticized and thus more susceptible to higher elongations than their respective more crystalline films (low-temperature process) because the former do not have many constraints, since the number of triple-stranded crystallites is rather limited compared to the latter. These results are in satisfactory agreement with findings reported elsewhere (Bradbury and Martin, 1952; Kellaway et al., 1978). The initially suggested Eqs. (7) and (8) for composite materials were also applied to chitosan/gelatin blends in order to calculate the estimates for the upper and lower limits of the tensile modulus (Ashby and Jones, 1985; Arvanitoyannis and Psomiadou, 1994; Arvanitoyannis et al., 1995):

$$E_{\text{blend}} = V_{\text{chitosan}} E_{\text{chitosan}} + (1 - V_{\text{chitosan}}) E_{\text{gelatin}} \tag{7}$$

$$E_{\text{blend}} = 1/\left(\frac{V_{\text{chitosan}}}{E_{\text{chitosan}}} + \frac{1 - V_{\text{chitosan}}}{E_{\text{gelatin}}}\right) \tag{8}$$

where E is the tensile or flexural modulus and V is the volume fraction of the respective component in the blend. If the values $E_{\text{chitosan}} = 2450$ MPa (Urbanczyk et al., 1994) and $E_{\text{gelatin}} = 800$ MPa (Fakirov et al., 1996) for 5%

Table 3
Mechanical properties and water vapour permeability (WVP) of chitosan/gelatin(1:1)/glycerol and chitosan/gelatin(1:1)/sorbitol blends, prepared by low- and high-temperature processes and conditioned at different relative humidities. The results give the average and the standard deviation of at least eight measurements

				Tensile strength (MPa)	Tensile modulus (MPa)	Elongation (%)	WVP ($g\ m^{-1}\ s^{-1}\ Pa^{-1}$) $\times 10^{-11}$
Low-temperature process							
Chitosan	Gelatin	Glycerol	Water				
47.5	47.5	0	5	130 ± 12	2050 ± 200	4.1 ± 0.5	0.07 ± 0.01
45.0	45.0	5	5	114 ± 10	1890 ± 150	7.5 ± 0.8	1.1 ± 0.2
40.0	40.0	15	5	83 ± 9	1760 ± 160	24.8 ± 2.3	3.7 ± 0.5
Chitosan	Gelatin	Sorbitol	Water				
45.5	45.5	4	5	106 ± 9	1850 ± 160	8.9 ± 1.1	1.4 ± 0.1
40.5	40.5	14	5	75 ± 8	1500 ± 150	27.5 ± 2.3	3.8 ± 0.2
High-temperature process							
Chitosan	Gelatin	Glycerol	Water				
47.5	47.5	0	5	111 ± 13	1700 ± 200	3.2 ± 0.5	0.15 ± 0.0012
45.0	45.0	5	5	94 ± 8	1450 ± 120	6.0 ± 0.8	1.3 ± 0.1
39.5	39.5	16	5	75 ± 6	1200 ± 130	39.6 ± 4.2	5.0 ± 0.3
Chitosan	Gelatin	Sorbitol	Water				
44.5	44.5	6	5	77 ± 9	1300 ± 140	6.5 ± 0.7	1.6 ± 0.2
40.0	40.0	15	5	68 ± 7	1050 ± 90	38.7 ± 3.5	6.3 ± 0.4

Table 4
Gas permeability (O_2, N_2, CO_2 in $cm^2\,s^{-1}\,Pa^{-1}$) of chitosan/gelatin(1:1)/glycerol and chitosan/gelatin(1:1)/sorbitol blends, prepared with low- and high-temperature processes and exposed to different relative humidities. The results give the average of at least five measurements at ambient temperature (23 ± 2°C)

				O_2	N_2	CO_2
Low-temperature process						
Chitosan	Gelatin	Glycerol	Water			
47.5	47.5	0	5	$1.3(\pm 0.2) \times 10^{-15}$	$2.4(\pm 0.5) \times 10^{-16}$	$5.6(\pm 0.3) \times 10^{-15}$
45.5	45.5	4	5	$3.0(\pm 0.4) \times 10^{-15}$	$4.1(\pm 0.1) \times 10^{-16}$	$8.6(\pm 0.2) \times 10^{-15}$
39.0	39.0	17	5	$6.4(\pm 0.5) \times 10^{-14}$	$1.4(\pm 0.2) \times 10^{-14}$	$2.4(\pm 0.5) \times 10^{-13}$
Chitosan	Gelatin	Sorbitol	Water			
45.0	45.0	5	5	$1.5(\pm 0.4) \times 10^{-15}$	$4.8(\pm 0.3) \times 10^{-16}$	$8.3(\pm 0.4) \times 10^{-15}$
40.5	40.5	14	5	$8.1(\pm 0.5) \times 10^{-14}$	$1.9(\pm 0.3) \times 10^{-14}$	$3.5(\pm 0.4) \times 10^{-13}$
High-temperature process						
Chitosan	Gelatin	Glycerol	Water			
47.5	47.5	0	5	$1.7(\pm 0.2) \times 10^{-14}$	$2.5(\pm 0.2) \times 10^{-15}$	$6.5(\pm 0.5) \times 10^{-14}$
45.5	45.5	4	5	$3.4(\pm 0.3) \times 10^{-14}$	$4.4(\pm 0.1) \times 10^{-15}$	$9.0(\pm 0.7) \times 10^{-14}$
40.0	40.0	15	5	$2.0(\pm 0.5) \times 10^{-13}$	$3.7(\pm 0.3) \times 10^{-14}$	$8.2(\pm 0.5) \times 10^{-13}$
Chitosan	Gelatin	Sorbitol	Water			
46.0	46.0	3	5	$5.3(\pm 0.3) \times 10^{-14}$	$9.2(\pm 0.8) \times 10^{-15}$	$2.1(\pm 0.5) \times 10^{-13}$
40.5	40.5	14	5	$1.6(\pm 0.1) \times 10^{-12}$	$2.4(\pm 0.2) \times 10^{-13}$	$4.3(\pm 0.4) \times 10^{-12}$

moisture content in chitosan/gelatin (1:1), the estimates obtained are 1625 and 1206 MPa, from Eqs. (7) and (8), respectively. The two estimates coincide because the tensile moduli for gelatin and chitosan are almost identical. The experimentally determined values (Table 3) are in satisfactory agreement with the above estimates.

Of course, the plasticization extent of the chitosan/gelatin blend depends on the nature of plasticizer; sorbitol has a more marked plasticizing effect (lowering the tensile strength and modulus) than glycerol, whereas the elongation of the two plasticized blends does not vary considerably.

3.2.2. Water vapour permeability (WVP)

Most edible films, apart from waxes and modified natural polymers, are described by a high WVP which, very often, makes them inappropriate for several applications unless a combination of two or more of them is employed (Torres, 1994). WVP is considered a rather crucial issue because most natural biopolymers are very prone to plasticization of water and the latter tends to cluster within the polymer matrix; protein/polysaccharide, protein/protein or polysaccharide/polysaccharide blends (Arvanitoyannis et al., 1996, 1997a, b, c; Schult and Paul, 1996; Psomiadou et al., 1996; Gennadios and Weller, 1990; Gennadios et al., 1993a, b, c, d, e). Table 4 shows that an increase in TPC (glycerol/sorbitol and water) is directly proportional to an increase in WVP in agreement with previous findings (Arvanitoyannis et al., 1997a, b, c).

3.2.3. Gas permeability (GP)

Interactions between packaging materials and foodstuffs can affect food quality. The required protection of the foodstuffs may be achieved with a single layer or a multilayer gas/water vapour barrier. Since gas permeability of food packaging materials is of great importance for food preservation, various experimental techniques such as volumetric, gravimetric and differential have been suggested for assessing the potential of packaging materials (Jasse et al., 1994). Prediction of gas barrier properties of synthetic polymers has long been a crucial issue in terms of prolonging the shelf life of foods, and several attempts have been made in this direction (Salame, 1986; van Krevelen, 1990; Bicerano, 1996). Salame (1986) assumed that the chemical groups or segments (i.e. –OH, $–CH_2–$, $–CH_3$) into which a polymer chain could be broken could be assigned a numerical value to predict permeability. Although this methodology repeatedly proved to be quite successful for synthetic polymers, it completely failed in the case of natural biopolymers. In 1978, Hopfenberg and Paul published their contribution on transport phenomena in polymer blends (quoted by Jasse et al., 1994) and described the blend gas permeability by various mathematical models. A linear semi-logarithmic behaviour was previously shown to provide a good fit for experimental permeability values in the case of completely miscible blends:

$$\ln P = \varphi_1 \ln P_1 + \varphi_2 \ln P_2 \tag{9}$$

where φ_1 and φ_2 are the volume fractions of components 1 and 2, respectively. For a 50/50 chitosan/gelatin blend, if we substitute the respective permeability values for CO_2 permeability ($P_{\text{chitosan}} = 1.7 \times 10^{-14}$ and $P_{\text{gelatin}} = 3.1 \times 10^{-15}\,cm^2\,s^{-1}\,Pa^{-1}$) for 5% moisture content we get $7.26 \times 10^{-15}\,cm^2\,s^{-1}\,Pa^{-1}$ which is in agreement with our findings (Table 4).

Table 4 shows that the mobility of the polymer matrix increases proportionally to the TPC. Fig. 4 is an example of an indirect T_g determination (gas permeability versus the inverse temperature). This change in the slope can be explained as follows; the initially localized water over selected sites becomes more homogeneously widespread and of high mobility after swelling of the polymeric matrix at high water content. Furthermore, the chitosan and the

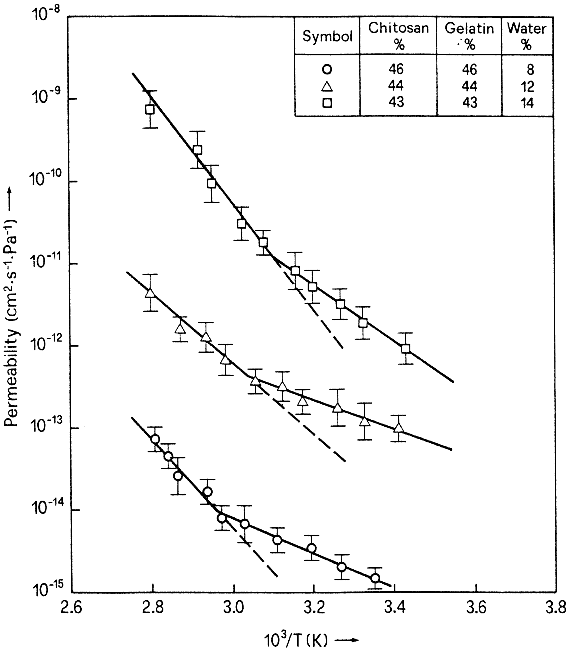


Fig. 4. Permeability of CO_2 in chitosan/gelatin/ water blends versus the inverse temperature (1/*T*). The results give the average and the standard deviation of at least six measurements.

gelatin chains move apart because the number of both intra- and interchain hydrogen bonds decreases proportionally to the water content. Therefore, plasticization of the polymer composite matrix leads to widening of the latter thus facilitating gas permeation in agreement with previous findings for gelatin, chitosan and starch-based blends (Arvanitoyannis and Biliaderis, 1998a, b; Lieberman and Guilbert, 1973; Arvanitoyannis et al., 1997a, b, c; Arvanitoyannis and Blanshard, 1993).

4. Conclusions

Polyol plasticized edible films consisting of chitosan and gelatin were prepared by casting at 60°C and evaporation at 22 or 60°C (low- and high-temperature methods, respectively). The films obtained by the low-temperature method were characterized by higher molecular ordering and crystallinity (renaturation as determined with DTA) compared to those of the high-temperature method. Furthermore, the low-temperature films were also endowed with higher tensile strength and modulus, higher activation energies for diffusion and permeation and lower gas and water vapour transmission rate. In general, the presence of a polyol as a plasticizer in the chitosan/gelatin blends was found to impart an enhanced mobility to the polymer blend mirrored by lowering of T_m, T_g and mechanical strength and higher gas/water permeation rates proportional to the total plasticizer content.

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