JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 5 (1994) 877–881

Intermolecular interactions in blends of hyaluronic acid with poly(vinyl alcohol) and ethylene-vinyl alcohol copolymers

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Films from blends of hyaluronic acid with poly(vinyl alcohol) and with ethylene-vinyl alcohol copolymers were prepared from solutions in water and dimethylsulfoxide by a casting method. The study of the interactions between the natural and synthetic component has been carried out through thermal and morphological analysis. The results show that the crystallinity of poly(vinyl alcohol) and its copolymers decreases with increasing hyaluronic acid content and these crystallizable polymers exhibit no tendency to crystallize when hyaluronic acid content is more than 80 wt%. The relatively good miscibility in these blends may be due to the capacity of the natural and synthetic component, each with abundant hydroxyl groups, to interact mutually through hydrogen bonding. The presence of such interactions was supported by the observation of the melting point depression of the synthetic component in thermal analysis of the blends. Melting point depression was more accentuated in melt recrystallized blends. These results suggest that the interactions between the two polymers are enhanced if the blends are brought close to the melting point of the synthetic component.

1. Introduction

The study of the interactions between natural and synthetic polymers has received little attention until quite recently. The great success obtained over the last few years in forging a closer relationship between life and materials sciences in order to use the great potentiality of the biological world for the realization of the new materials, outlined the role played by the above mentioned interactions.

Hyaluronic acid has been used as a biological component in preparing "bioartificial" [1] blends in which the synthetic component chosen from commercially available polymers such as poly(vinyl alcohol) and ethylene-vinyl alcohol copolymers. Hyaluronic acid is a naturally occurring mucopolysaccharide consisting of residues of D-glucuronic acid and N-acetyl-D-glucosamine. It is found in cartilage, in eye vitreous humour, umbilical cord and synovial fluid [2]. Poly(vinyl alcohol) and its copolymers are already in use in the biomedical field due to their hydrophilic properties and high biocompatibility.

The purpose of the present work is the preparation of HA/PVA and HA/Clarene blends and the evaluation of intermolecular interactions between the components mainly through differential scanning calorimetry (DSC) and scanning electron microscopy (SEM) analysis. Both biological and synthetic polymers used are capable of forming intra and intermolecular hydrogen bonds between hydroxyl groups. Therefore, for the present systems, it is also of interest to ascertain whether molecular interactions based on hydrogen bonding take place between the two different polymers, and contribute to the enhancement of the miscibility of the two components.

2. Experimental procedures

2.1. Materials

Hyaluronic acid (HA) in the form of sodium salt was obtained from FAB S.p.A., Italy. PVA (powdered) with an average molecular weight of 114 000 (determined by the viscosimetric method) and a hydrolysis degree of 100%, was purchased from Aldrich Chemie, Steinheim, Germany. The ethylene-vinyl alcohol copolymers (Clarene®, Solvay & Cie, Italy) used in this work are the following:

- Clarene® L6 (low ethylene content: 29 mol %)
- Clarene® P10 (moderate ethylene content: 36 mol %)
- Clarene® R20 (high ethylene content: 40 mol %).

2.2. Preparation of samples

HA/PVA: PVA solutions (1% w/v) and HA solutions (1% w/v) were prepared in water. These solutions were mixed in appropriate proportions, so that the relative composition of the two polymers in the mixed solutions ranged from 10/90 to 90/10 in weight per cent ratio, the first numeral referring to HA content throughout this work. The resulting blends were used for film preparation by casting at about 60 °C. HA and PVA homopolymer films were also obtained by the same method. The films were stored in a desiccator until used.

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HA/Clarene®: a common solvent for ethylene-vinyl alcohol copolymers is the dimethylsulfoxide (DMSO), so Clarene® solutions were prepared in DMSO (1% w/v). A HA solution in DMSO (1% w/v) was obtained by substitution of solvent (H_2O). The solutions obtained were mixed in the desired proportions: weight percentage of HA ranged from 10 to 90. The blends realized are the following: HA/Clarene L6, HA/Clarene P10, HA/Clarene R20. The films were obtained by casting at about 60 °C, and stored in a desiccator until used.

2.3. Apparatus and techniques

Differential scanning calorimetry (DSC) measurements were performed with a Perkin-Elmer DSC-7, in a N_2 atmosphere. The instrument was calibrated with an indium standard. The thermal properties of the blends and homopolymers were analysed in three scans: namely, the first heating, second heating, and cooling scan. The first heating scan was carried out for characterizing solution-cast films at a rate of 10 °C/min from 30 °C up to about 10 °C above the melting temperature of the synthetic components. In this scan, however, the thermograms were disturbed in the range 60–150 °C due to the presence of trace amounts of solvent. Thus only the melting point behaviour will be reported for the solution-cast samples. The second heating scan, within the same temperature range, was carried out after a quick cooling (200 °C/min), in order to characterize melt-crystallized samples. An additional cooling scan was carried out as follows: the samples were heated up to the melting point of the synthetic polymer and were kept at that temperature for 2 min and then cooled at a rate of 20 °C/min to record crystallization. The calorimetric melting temperature, T_m, and the apparent enthalpy of fusion, ΔH_f, of each sample were determined from the maximum and the area of the melting peak, respectively. In same manner, the crystallization temperature, T_c, and the heat of crystallization, ΔH_c, were also evaluated from the exothermic peak in the cooling cycle.

SEM analysis was performed with a Jeol T 300 scanning electron microscope. Films were freeze fractured in liquid N_2 and sputter-coated with gold.

3. Results and discussion

3.1. DSC characterization

Since the HA homopolymer films prepared by casting showed no significant transition in the explored temperature range, it was interesting to estimate how the thermal transitions of PVA and Clarene varied with the composition of the blends. The thermodynamic values obtained through the analysis of the DSC curves are listed in Table I.

(a) Solution-cast samples (first heating). The thermograms obtained in the first heating of the solution-cast films are disturbed by the presence of solvent in the range 60 to 150 °C. Thus in some cases only the melting temperature may be precisely estimated. The experimental melting points T_m and ΔH_f corresponding to PVA, Clarene are given at the left-side of Table I. These data show a depression of the melting point of the synthetic polymer when HA content increases. A simultaneous reduction of the fusion heat indicates that the degree of crystallinity of PVA and Clarene decreases due to blending with HA.

(b) Melt-crystallized samples (second heating). In this case no perturbing effect due to the solvent is present, so it is possible to estimate the composition dependence of both the melting point and the fusion heat for blends that underwent the same thermal history. The experimental T_m and ΔH_f corresponding to PVA and Clarene in the second scan (Table I) show an important depression of the melting temperature of the synthetic polymer as HA increases. A simultaneous decrease of the fusion heat indicates that the crystallinity of the synthetic polymer decreases with increasing HA, and is completely suppressed when HA exceeds 70–80 wt %.

The T_m depression is more marked for the melt-crystallized samples than for the solution-cast samples. This suggests that some kind of interaction between the two polymers becomes more prominent at elevated temperatures near the T_m of the synthetic polymer. Such similar mixing effects were observed in the crystallization behaviour, as can be seen at the right-side of Table I. The systematic decrease of the crystallization temperature T_c and the exothermic area with increasing HA content is observed. For weight fractions of HA above 0.5, the crystallization exothermic event is not detectable from DSC curves.

Similar depression phenomena have been found in several polymeric blends exhibiting good miscibility: blends of poly(vinylidene fluoride) (PVF2) with poly(methyl methacrylate) (PMMA) [3], with poly(ethyl methacrylate) (PEMA) [4, 5] and with other amorphous polymers [6]; blends of poly(vinyl alcohol) (PVA) with poly(acrylic acid) (PAA) [7], and so on [8, 9]. Nishi and Wang [3] showed that the melting point depression phenomena of PVF2 observed in the blends with PMMA was explicable in terms of thermodynamic mixing accompanied by an exothermic interaction between a crystalline polymer and an amorphous polymer. The same thermodynamic approach was used by Paul *et al.* [5, 6, 8, 10] and several other workers [4, 11–13]. A form of the equation for the melting point depression in crystalline/amorphous polymer blends can be written as follows [5, 10]:

$$\Delta T_m = T_m^0 - T_m = T_m^0(V_{2u}/\Delta H_{2u})Bv_1^2$$

where the subscripts 1 and 2 are used to designate the amorphous and crystalline polymer components, respectively, T_m^0 is the melting point of the pure crystalline polymer 2, T_m is the melting point of the mixture, v is the volume fraction, V_u is the molar volume of the repeating units, ΔH_u is the enthalpy of fusion per mole of repeating unit, and B refers to the interaction energy density of the two polymers, in practice, related to the thermodynamic interaction parameter χ_{12} by:

$$B = RT_m^0(\chi_{12}/V_{1u})$$

where R is the gas constant. In accordance with the above equation, we attempted to make such plots

TABLE I Melting temperature T_m, heat of fusion ΔH_f crystallization temperature T_c and heat of crystallization ΔH_c of HA-based blends, measured by DSC

	1st Heating		2nd Heating		Cooling	
	T_m °C	ΔH_f J/g[a]	T_m °C	ΔH_f J/g[a]	T_c °C	ΔH_c J/g[a]
HA/PVA (w/w)						
0/100	224.5	78.2	225.4	66.2	187.6	50.3
10/90	223.9	71.1	221.6	61.3	166.3	45.5
20/80	222.8	68.2	217.1	52.4	165.1	31.0
30/70	221.1	51.1	209.3	49.0	163.2	28.3
40/60	220.2	48.2	201.4	45.2	140.8	6.8
50/50	218.1	NE	195.5	40.2	NE	~ 0
60/40	NE		~ 183.4	NE	NE	
70/30	NE		NE		ND	
80/20	ND		ND		ND	
90/10	ND		ND		ND	
HA/Clarene L6						
0/100	182.3	64.6	181.7	55.5	165.0	59.4
10/90	175.1	58.2	180.5	53.0	163.3	52.2
20/80	173.4	44.7	179.5	51.3	161.2	56.2
30/70	168.9	37.4	178.3	48.6	159.2	43.7
40/60	167.2	36.4	175.6	45.6	158.2	40.9
50/50	160.9	NE	170.4	35.6	156.0	34.2
60/40	NE		166.4	30.9	151.5	24.1
70/30	NE		161.3	26.8	142.8	20.2
80/20	ND		ND		~ 128	10.2
90/10	ND		ND		ND	NE
HA/Clarene P10						
0/100	172.7	62.1	175.2	56.7	152.2	59.6
10/90	166.5	49.5	169.8	53.4	150.4	53.6
20/80	163.3	48.9	167.7	48.9	149.2	48.5
30/70	162.1	40.1	166.5	47.1	147.7	43.7
40/60	160.0	36.1	165.3	38.9	144.3	38.2
50/50	158.2	30.2	164.8	30.1	137.2	30.4
60/40	153.1	NE	160.5	28.9	~ 120.1	~ 0
70/30	152.1	NE	156.8	22.9	NE	
80/20	NE		ND		ND	
90/10	NE		ND		ND	
HA/Clarene R20						
0/100	161.8	57.6	164.9	50.5	142.2	54.1
10/90	156.6	49.2	161.2	44.7	137.3	50.2
20/80	155.3	44.1	160.5	41.6	134.3	56.2
30/70	149.9	32.8	153.1	38.3	132.5	38.5
40/60	136.5	25.2	147.1	30.1	129.2	5.1
50/50	~ 120	NE	141.3	25.0	~ 104.4	NE
60/40	NE		130.9	12.3	~ 85	NE
70/30	NE		~ 122.7	~ 4.5	NE	
80/20	ND		ND		ND	
90/10	ND		ND		ND	

NE = could not be estimated. ND = not detected.

[a] Based on weight of PVA or of Clarene.

using the reproducible melting data of HA/PVA and HA/Clarene blends obtained in the DSC study (second scan). As a result the relations given in Fig. 1a–d were obtained, where the solid lines were drawn by the least-squares method assuming a linear relationship between ΔT_m and v_1^2. Small intercepts are observed: they can be assigned to residual entropic effects [5, 6, 10]. The slope of these lines calculated by linear regression gives B values for HA/PVA and HA/Clarene blends. From these B values it is possible to calculate the χ_{12} polymer interaction parameters.

The values 1.52 g/cm^3 [14], 1.19 g/cm^3 [15] 1.17 g/cm^3 [15], 1.16 g/cm^3 [15] were used as specific density of HA, Clarene L6, Clarene P10 and Clarene R20, respectively, to convert weight fractions into volume fractions. The value of 1.6 Kcal/mol is reported in the literature for ΔH_{2u} corresponding to the 100% crystalline PVA [16]. Unfortunately, there are no available literature values of the latent heat of fusion of the 100% crystalline Clarenes, ΔH_{2u}, to our knowledge. It is thus impossible precisely to assess the value of the parameter B for the HA/Clarene blends. As a first estimation, however, taking into account that the X-ray analysis of fine powder Clarene samples showed that the crystallinity of ethylene-vinyl alcohol copolymers used in this work was estimated to range

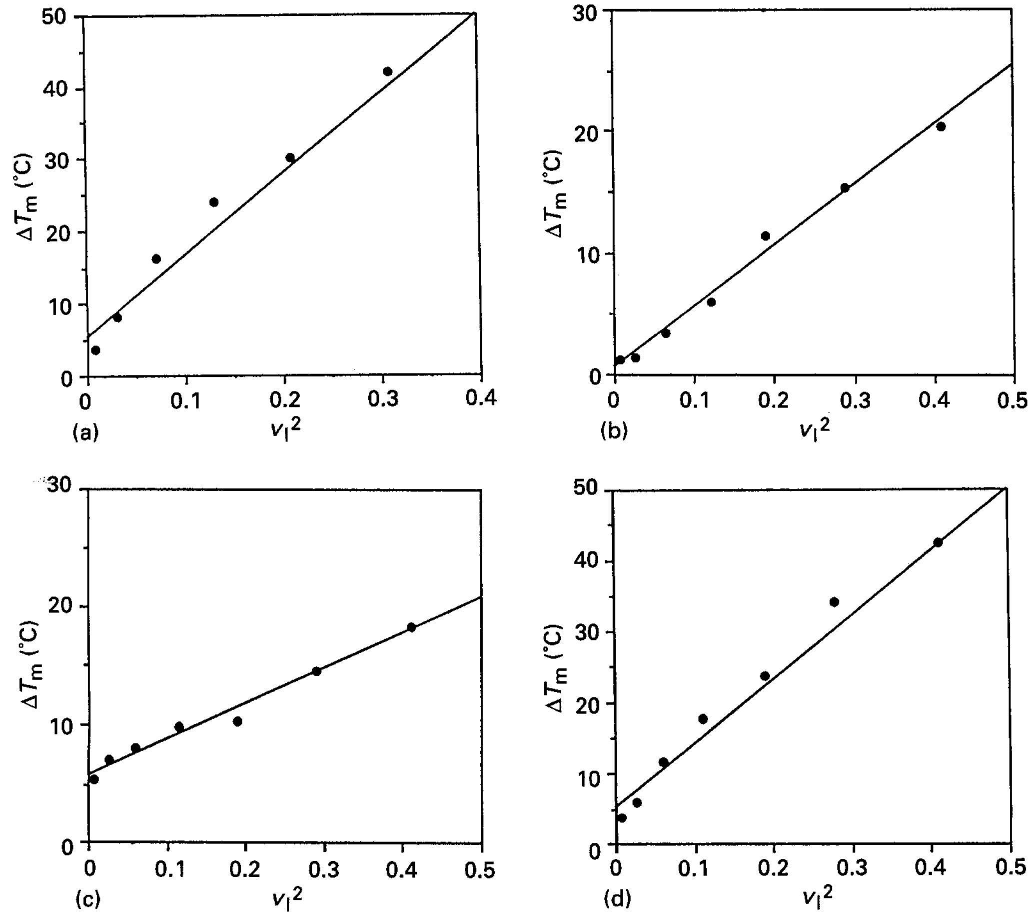

Figure 1 Melting point depression of (a) PVA, (b) Clarene L6 (c) Clarene P10, (d) Clarene R20 in HA-based blends as a function of volume fraction of HA.

between 30 and 50%, 15 and 30% and 25 and 40% for Clarene L6, Clarene P10 and Clarene R20, respectively, and that the ΔH_f values for these copolymers have been obtained experimentally, a range for the interaction energy density B may be estimated. B and χ_{12} values calculated are given in Table II. These high negative values obtained for B and χ_{12} are characteristic of very strong intermolecular interactions between molten synthetic polymer and amorphous HA molecules. These values can be compared with other values for H-bonded blends (Table III). However, it is necessary to remember that a melting point depression may also originate from morphological effects [3, 5, 9, 17, 22–24], such as the lowering of the perfection and/or size of the crystals, because of the presence of the amorphous component in the blend. The contribution of such morphological effects may be examined by using samples isothermally crystallized at various temperatures from the molten state [17, 24].

TABLE II Interaction energy density and parameter of HA-based blends

Blend	B (cal/cm^3)	χ_{12}
HA/PVA	$-11.2 \rightarrow -9.1$	$-2.7 \rightarrow -2.2$
HA/Clarene L6	$-5.7 \rightarrow -3.4$	$-1.5 \rightarrow -0.9$
HA/Clarene P10	$-6.9 \rightarrow -3.8$	$-1.6 \rightarrow -1.1$
HA/Clarene R20	$-6.6 \rightarrow -5.9$	$-1.9 \rightarrow -1.6$

TABLE III Interaction energy density and parameter for various binary blends with specific interactions

Blend	B (cal/cm^3)	χ_{12}
PAA/PVA1 [7]	-19.9	-1.24
PVP/PVA1 [16]	-7.6	-0.69
PVF2/PMMA [17]	-2.1	-0.2
PVF2/PEMA [18]	-2.8	-0.34
PS/PPO [19]	-10.4	-0.17
PCL/Saran [20]	$-12.2 \rightarrow -5.0$	$-1.06 \rightarrow -0.55$
Cellulose/PVA1 [21]	$-18 \rightarrow -10$	$-1.9 \rightarrow -1.1$

3.2. Optical and SEM characterization

Films of homopolymers prepared by casting are transparent. The HA/PVA films are transparent over the whole composition range. HA/Clarene films show an increasing opacity when the copolymer content increases. For HA/PVA films the SEM analysis did not show evident phase separation over the whole composition range. HA/Clarene films showed uniform and homogeneous structures for high content of HA, and less uniform for low HA content. These observations are not, by themselves, proof of miscibility but appear to support the data obtained by thermal analysis.

4. Conclusions

Films of HA/PVA and HA/Clarene can be easily prepared over the whole composition range from solutions in water and DMSO, respectively, by the casting method described in this work. The DSC study revealed that the PVA and Clarene crystallinity decreases with increasing HA content, and at HA contents more than 70%, the synthetic component exhibits no tendency to crystallize. At 80% or more of HA content, the blends seem to exhibit a high level of miscibility in the abundant amorphous regions. It is presumed that an interaction based on hydrogen bonding between the hydroxyl groups of the two polymers aids in the attainment of the relatively good miscibility. Indeed, in the case where the concentration of hydroxyl groups is higher (PVA) the interactions are more evident. The melting point depression of the crystalline components observed in the DSC measurement (first scan) supports the presence of such an interaction. Depressions are also observed more notably in the melting temperatures of the melt-crystallized blends. It is suggested that the thermodynamic interactions between the two polymers may be enhanced by increasing temperature above the melting point of the synthetic polymer. The contribution of some kind of morphological effect remains to be elucidated.

Acknowledgements

Thanks are due to P. Narducci for performing SEM analysis. This work has been partially supported by Italian C.N.R.

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